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THE FUNCTION OF VITAMIN E*

ERNEST BEERSTECHER, JR., AND ELEANOR J. EDMONDS

It is now over twenty years since the first attempts were made to assess the function of vitamin E in animal metabolism. Despite numerous studies designed to clarify this problem, there is at present little more that the general impression that "vitamin E acts primarily and specifically through *some* enzyme system and secondarily in a non-specific manner as a physiological antioxidant" (Ames and Risley). The great variety of biochemical lesions that have been reported in vitamin E deficient animals, including such things as decreased muscle creatine, creatine phosphorylation, glutamine, and transaminase activity; creatinuria, aminoaciduria, and enhanced oxygen consumption of E deficient muscle, coupled with the reported synergistic effects of vitamin E and vitamin B_6 deficiencies (MacKenzie), have led some workers to question whether the tocopherols do have any function other than their antioxidant activity. Many of the lesions observed may well be explained on the basis of a generalized tissue breakdown followed by various secondary metabolic defects. This fact, together with the need for an analytical method for vitamin E comparable in sensitivity to the microbiological methods for B vitamins, would seem at present to be a serious detriment to further progress in this field.

It has been pointed out from time to time that substances actively involved in the fundamental metabolic processes are more subject to destruction in biological systems reacting at higher rates, while this is not necessarily true of substances which function merely in a buffering or regulatory capacity. There is thus a correlation between the basal metabolic rate of various organisms and their nutritional requirements for the B vitamins (Beerstecher; Williams *et al.*) but not other vitamins, minerals, etc. The determination of whether or not the requirement for a substance *per unit of body weight* increases with decreasing total body weight for a number of

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species may thus be applied as a sort of test of the functional nature of a dietary component, since the size of a species tends to vary inversely with its basal metabolic rate. A rearrangement of data recently presented (Harris) on the requirements for vitamin E has consequently been made. The data are shown in Figure I. It will be seen that a tendency

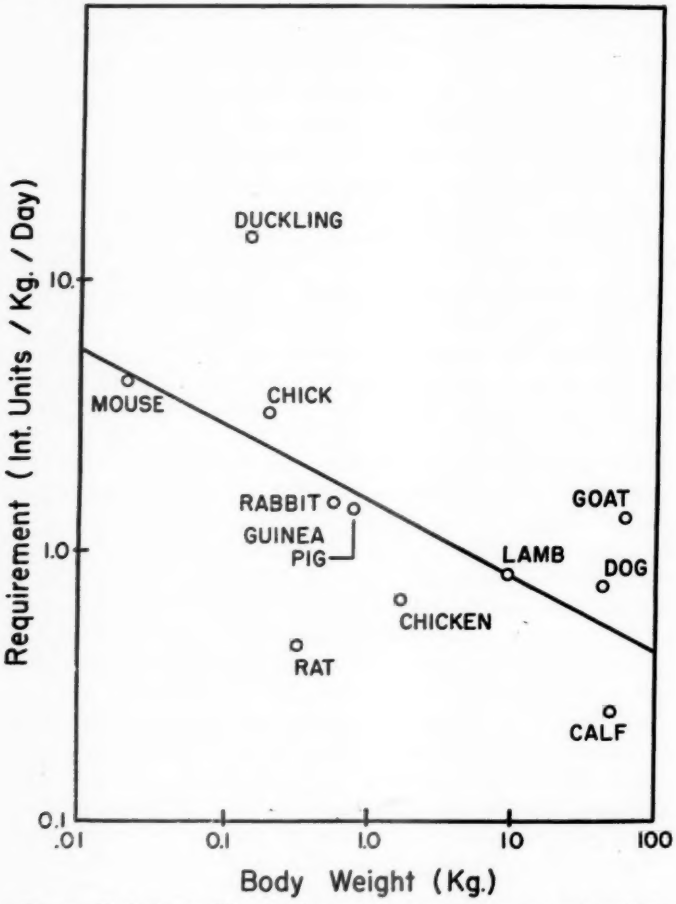

FIG. 1. Variation in Vitamin E requirements per day per unit of body weight with total body weight for a number of species.

toward increased requirements per unit of body weight with decreased species size appears to exist as has previously been implied (Harris). A better correlation could not be expected in view of the approximate nature of the values for the requirements which were assessed by various deficiency criteria. It is also of considerable interest that the major deviations above and below the line represent extremely high and low fat bearing species respectively. Such an observation might be expected in view of the dual roles suggested for the tocopherols.

It has recently been pointed out (Williams *et al.*) that there is some resemblance in the distribution of the B vitamins and vitamin E, although numerous exceptions do occur. These exceptions may of course be reflections upon the sensitivity of present analytical methods for tocopherols. The data in Figure I suggest that vitamin E is actively involved in the metabolic processes in a manner analogous to that in which the B vitamins function and preliminary results obtained in these laboratories suggest that the function may be closely associated with the tryptophan-vitamin B_6 interrelationship. Work along these lines is now in progress.

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ALTERATION OF PITUITARY ASCORBIC ACID BY SEVERE STRESS*

JOSEPH S. BIERMAN, MELVIN HESS, AND JOHN C. FINERTY

High concentrations of ascorbic acid are known to be present in the adrenal glands, in corpora lutea and in the pituitary gland. Investigations of the adrenals by Sayers and Sayers (1947), Long (1947), and others and of the ovaries by Miller and Everett (1948) have established ascorbic acid as a constituent involved in secretory activity, but little is known of its action in the hypophysis. Pituitary ascorbic acid concentration has been reported by Phillips and Chang (1934) in rats subjected to experimental diets and flourine poisoning. They found a range of 120 to 595 mg. ascorbic acid per 100 gm. pituitary tissue, but it is difficult to determine a normal level from their work since a variety of diets were used, with and without added NaF. More than half the rats in each group were treated with vital dyes prior to autopsy.

In young female rats (81 grams), Salhanick, Zarrow, and Zarrow (1949) found 112 mg. per cent ascorbic acid in the pituitary gland, whereas in older rats (171 and 216 grams), the concentration increased to 131 mg. per cent and 145 mg. per cent, respectively. They found no significant changes in the concentration of pituitary ascorbic acid under various experimental conditions designed to alter the rate of secretion of ACTH, thyrotrophin, gonadotrophins or posterior pituitary factors. However, Poumeau-Delille (1950) found a normal level of 110 mg. per cent in intact rats and demonstrated an increase to 150 mg. per cent for several weeks following adrenalectomy, during which the rats were maintained on 1 per cent NaCl drinking water.

The purpose of the present paper is to establish the normal level of ascorbic acid in the rat pituitary gland and to demonstrate changes in ascorbic acid concentration with alterations in secretory activity.

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Materials and Methods

Male rats obtained from the Holtzman-Rolfsmeyer Company were used in all experiments. They were anesthetized with sodium pentobarbital and the entire pituitary gland removed immediately, weighed on a torsion balance and placed in a flask containing 6% trichloracetic acid. Ascorbic acid was determined by the method of Roe and Kuether (1943). For the most part, chemical determinations were done on the combined ascorbic acid of two pituitaries, since this concentration gave more reliable readings on the colorimeter scale.

Secretory activity of the hypophysis was modified by adrenalectomy, sham adrenalectomy, epinephrine injection and exposure to high temperature (36° C.).

Results

Normal level of pituitary ascorbic acid: It was found that practically all the ascorbic acid of the rat pituitary is located in the anterior lobes, the posterior lobe having a negligible amount. Table 1 summarizes the normal level of ascorbic acid in the rat pituitary at various ages.

TABLE I

Ascorbic Acid Content of Pituitary Glands in Intact Rats

Age (days)	No. of rats	No. of determinations	Ascorbic Acid mg./100 gm.	Ascorbic Acid tissue
36–38	6	3	96.1	5.7*
40–41	18	9	94.7	3.8
40–45	9	5	96.2	2.5
68	4	2	99.3	1.7
76–79	20	10	104.2	3.8

*Standard error.

Experimental modification of pituitary ascorbic acid: Attempts to modify pituitary ascorbic acid levels by short periods of stress were unsuccessful. No significant change from the normal occurred following adrenalectomy of 2 hours duration; sham adrenalectomy; or intraperitoneal injection of 20 micrograms of epinephrine with autopsy ½, 1 and 3 hours later.

When six-week old male rats were subjected to bilateral adrenalectomy plus exposure to high temperature (36° C.)

for 24 hours there was a marked decrease in pituitary ascorbic acid. Similar results were obtained when rats were exposed to heat and received a total of 24 micrograms epinephrine in 4 divided doses during 5 hours prior to autopsy. Unoperated rats exposed to heat, and 24-hour adrenalectomized rats kept at 22° C. showed no deviation from the normal value.

Table 2 illustrates the results of an experiment to test the hypothesis that prolonged severe stress depletes pituitary ascorbic acid.

TABLE II

Effects of Severe Stress of 24 Hours Duration on Pituitary Ascorbic Acid

Procedure	No. of rats	No. of determinations	Ascorbic Acid mg./100 gm. tissue
Control (22°C.)	14	7	104.2 1.4*
Sham adrenalectomy (22°C.)	6	3	114.7 1.8
Adrenalectomy (22°C.) ...	10	5	105.9 5.3
Adrenalectomy (36°C.) ...	14	7	80.1 2.2

*Standard error.

It can be seen from these data that when young adult male rats were adrenalectomized and subsequently exposed to the additional stress of high temperature for 24 hours there was a 23% decrease in pituitary ascorbic acid concentration. No significant change occurred with adrenalectomy alone or with sham adrenalectomy.

Discussion

The failure to observe any change in pituitary ascorbic acid with mild stresses agrees with the results of Salhanick *et al.* (1949). Prolonged severe stress is necessary for alteration in pituitary ascorbic acid concentration in contrast to the rapid decrease in adrenal ascorbic acid following stress or ACTH administration. This correlates well with the observations of Sayers and Cheng (1949) who found that severe stress is required to modify ACTH synthesis in the hypophysis. The increased ascorbic acid content of the hypophysis found by Poumeau-Delille (1949) seems to disagree with the findings of Salhanick *et al.* (1950) and the present study. However, the difference in time of determination may explain the

divergent results. Salhanick *et al.* used cold for 1 hr. or 12 hrs., adrenalectomy for 24 hrs. or 72 hrs., thiouracil treatment (0.1%) for 12 weeks in rats, and electrical stimulation of the cervix, or vagus nerve in rabbits, without significant change in ascorbic acid concentration. Poumeau-Delille found the increase in periods of several weeks after adrenalectomy, and states that injection of desoxycorticosterone reduced ascorbic acid concentrations to normal levels. In a certain number of the latter experiments Poumeau-Delille mentions a temporary decrease shortly after adrenalectomy to 65 to 75 mg. per cent, which may correspond to that found in the present experiments after 24 hours of adrenalectomy and exposure to high temperature.

A fundamental difference exists between the pituitary and adrenal glands in that the former has a relatively high storage capacity whereas the latter stores relatively small amounts of its products. Since there is no stored hormone, and since large amounts of cortical hormones are secreted (Vogt, 1943; Ingle, 1944), excitation of adrenal cortical activity must stimulate rapid synthesis of secretory products. On the other hand, the early response of the hypophysis is release of stored hormone followed by synthesis after prolonged intense stimulation. Ascorbic acid decrease occurs rapidly after adrenal stimulation and is delayed after pituitary stimulation implying that it is associated with synthesis of secretory products rather than with their release from the gland.

The effectiveness of heat stress added to bilateral adrenalectomy in the present work may be attributed to more rapid utilization of cortical hormones in blood and tissues when severe stress was administered.

Summary

Normal ascorbic acid concentration in the young rat pituitary gland is approximately 100 mg./100 gm. of tissue. Pituitary ascorbic acid may be reduced by prolonged severe stress. It is suggested that ascorbic acid is associated with synthesis of hormones rather than with their release.

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A STILL FOR CONTINUOUS FRACTIONATION*

IBEN BROWNING AND LLOYD S. LOCKINGEN

In our laboratory, it is desirable to obtain water which has been triply-distilled, the last two distillations being done in Pyrex glass. In addition, for our convenience, we wished to make the still automatic.

To make an automatic still of reasonable magnitude for laboratory use, it was necessary to design a system capable of continuous feeding and fraction cutting. In order to obtain a design easy to fabricate, the still was so constructed that all joints except one are tee joints.

The complete mechanism consists of two distilling units, similar to that illustrated in Fig. 1; the first of these is fed with singly distilled water, and the second is fed by the middle cut from the first. Only the middle cut of the second still is saved. The principles of operation follow.

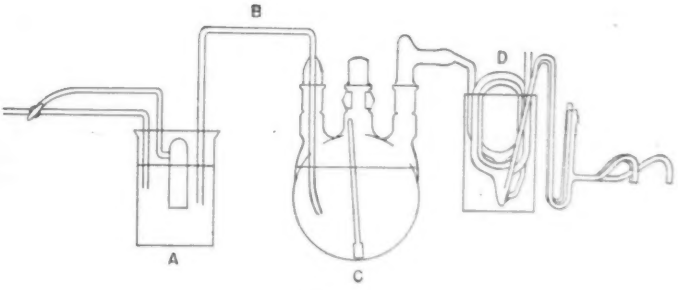

FIGURE 1

First Distillation Unit: Beaker *A* is fed with water from a distilled water line, and the level is kept approximately constant by a floater-stopcock arrangement. Siphon *B* carries distilled water from Beaker *A* to Flask *C* so that the water level in this flask remains approximately constant. Steam

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from Flask *C*, which is electrically heated, passes through the triple spiral condenser *D* which is surrounded by tap water supplied at a constant rate from a constant level tap water source. The cooling water in the condenser can flows from the last spiral (which condenses the last cut) toward the first spiral then out of the can through an exit port which maintains a constant water level. The condensate accumulates in the bottom of each of the three loops of the spiral and is removed by constant level siphons to the collecting point. The first and third cuts are discarded and the middle cut is collected in a container which is in turn connected to the next distillation stage.

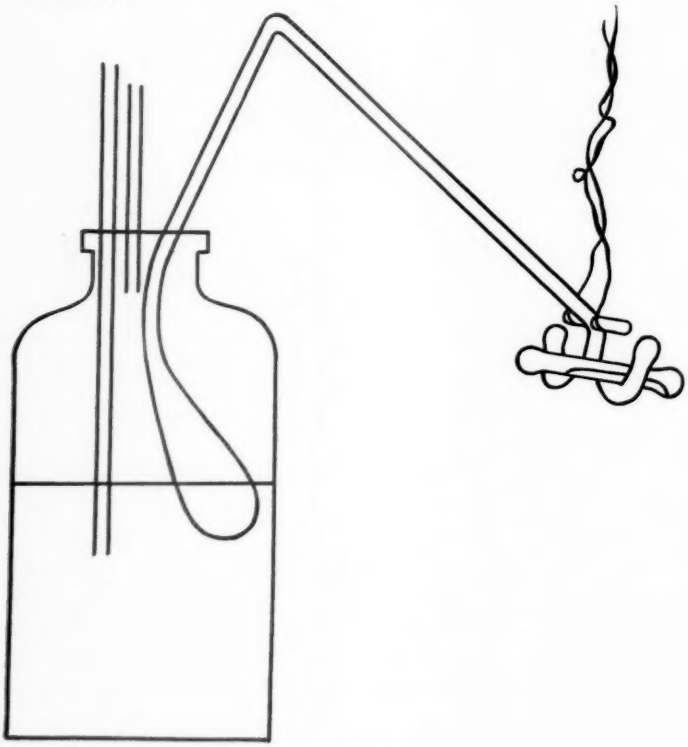

FIGURE 2

Second Distillation Stage: The novelty at this stage is a floater operated mercury switch (Fig. 2) operating in unstable equilibrium so that when the depth increases by approximately 2 cm. in the collection apparatus, the mercury switch is closed, activating a relay which in turn completes the circuit and transmits power to the second stage heating element. Distillation in this unit occurs until the level in the reservoir returns to a depth which permits the mercury switch to open and shut off power to the heater.

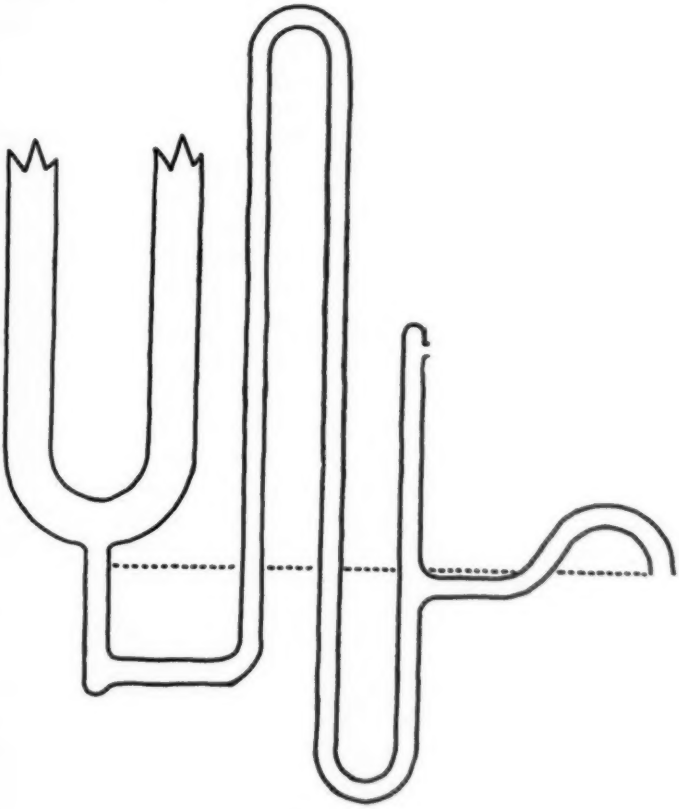

FIGURE 3

Fig. 3 shows the constant level siphon which is used to remove the condensate fraction over the top of the condenser can. One critical relationship exists in it, namely, that the condensate must drop free from the end of the siphon at a level below the main passageway in the spiral. If this drainage point were too high, free passage of steam in the spiral would be prevented. The other critical relationship of this apparatus is the steam pressure maintained within the boiling flask. This pressure must be quite low in order that siphon *B* can operate on a very small hydraulic head. Such a low pressure is possible only if condenser *D* is a free escape passage for steam.

Once each day the first boiling flask is siphoned empty in order to eliminate precipitate. A small quantity of potassium permanganate and sodium hydroxide are then added to it and it is permitted to fill itself and recommence operation. A 550 watt heating element under each distillation flask is found ample to produce almost one liter of triply distilled water per twenty-four hour day. The purity of the water is such that no detectable precipitate has formed in the last distillation flask over a period of several months. The pH of the collected water is approximately 6.4; its specific resistance in a standard cell is in excess of 300,000 ohms.

PSEUDOGLYCOSURIA FOLLOWING MASSIVE ANTIBIOTIC THERAPY*

RANDOLPH CLEMENTS AND G. A. EMERSON

Three years ago, we treated a group of some 150 mice with repeated massive doses of penicillin, procaine penicillin, or streptomycin before and after giving these animals varying single or repeated doses of alloxan. This work was suggested by evidence that penicillin may act to inhibit a mononucleotidase (Dufrenoy and Pratt, 1948) and that streptomycin may combine with nucleic acids or inhibit a depolymerase of nucleic acids of higher order, together with our belief (Emerson, Ewing, and Thomas, 1946) that the diabetogenic effect of alloxan may be related to a disturbance of nucleic acid metabolism.

Glycosuria was estimated with Benedict's solution as an indicator of diabetogenic effects in the treated mice, with urine freshly expressed from the bladder. Tests were made at intervals after intramuscular injection of 1–10 mega U./kg. of Na penicillin G, and at different times during a course of repeated daily doses of 1 mega U./kg. of Na penicillin G given intramuscularly for a maximum of 23 days and an average of 12.4 days to a group of mice. Other mice were given 2–10 mega U./kg. of Na penicillin G or of procaine penicillin G, in single or repeated doses, and were handled similarly.

As might be expected, animals not treated with alloxan but receiving large doses of procaine penicillin G more uniformly showed prolonged pseudoglycosuria than did those receiving similar massive doses of Na penicillin G, although a majority of the latter mice showed glycosuria at some time, even during periods of inanition. Other mice receiving streptomycin, aureomycin, aminopterin or other agents in doses approaching the respective LD_{50} of each agent did not regularly show the presence of significant amounts of reducing substances in their urine unless treated with alloxan also.

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*From the Department of Pharmacology and Toxicology, The University of Texas Medical Branch, Galveston. Received for publication July 5, 1951.

Since an apparent glycosuria occurred in mice treated with either form of penicillin, with or without alloxan treatment, and occasionally in mice treated with other agents, study of possible antidiabetogenic effects was abandoned without further tests of disturbances in carbohydrate metabolism, although pancreases and other tissues were fixed in Helly's fluid and dehydrated with dioxane for later histologic study by Professor T. B. Thomas of Carleton College.

Our interest was drawn to the possibility that an apparent glycosuria might occur clinically after large doses of antibiotics, by brief reports that this may occur with penicillin (Boldyreff, 1947) and streptomycin (Hilden and Møller, 1950). Urinary excretion of a non-glucose reducing substance has been reported (Beyer *et al.*, 1947) in dogs receiving large doses of caronamide. If a true glycosuria occurred after antibiotic therapy, it might have some significance in diseases of the genitourinary system, since glucose is said to potentiate antibacterial actions of penicillin (Lochhead and Timonin, 1939), while the purported direct inhibiting effect of glucose on the efficacy of streptomycin has been denied (Green and Waksman, 1948).

Accordingly, records of 55 patients in the John Sealy Hospital, all of whom had received massive antibiotic therapy for such conditions as subacute bacterial endocarditis, meningitides, or tuberculosis, were examined through the courtesy of Miss M. McArdle. Of these, only 2 showed apparent glycosuria. The first instance occurred in a 31-year-old white female with tuberculous meningitis, who showed + glycosuria on 6 occasions while receiving streptomycin intramuscularly and intrathecally; reducing substances were not estimated in blood or cerebrospinal fluid. The second patient, a 22-year-old white female, had acute meningococcic meningitis for which she received sulfonamides, penicillin, streptomycin and aureomycin; she showed a + + + glycosuria on one occasion and + glycosuria at another, but had a fasting blood sugar of 107 mg. %. Another patient, with tuberculous meningo-encephalitis treated with dihydrostreptomycin, showed a + glycosuria only before beginning therapy.

Hilden and Møller (1950) noted glycosuria in 2 patients receiving streptomycin intramuscularly, but consider this of

little clinical significance since dihydrostreptomycin, which in their experience does not give rise to glycosuria, is generally preferred for systemic therapy. Solutions of aureomycin and chloramphenicol show reducing power, but clinically do not cause pseudoglycosuria.

Hilden and Møller (1950) further indicate that high contents of reducing substances may be found in the cerebrospinal fluid of patients receiving streptomycin intrathecally; dihydrostreptomycin is rarely given by this route. Differences in pharmacologic and antigenic properties of streptomycin and dihydrostreptomycin (Waksman, 1949) indicate that there is little if any interconversion in the body, but it has also been proposed (Waksman, 1949) that dihydrostreptomycin owes its antibacterial activity to oxidation by microorganisms to streptomycin; the reported lack of pseudoglycosuria following treatment with dihydrostreptomycin supports other evidence that biological oxidation of this agent is not appreciable.

Boldyreff (1947) noted an apparent + glycosuria in an infant receiving 20,000 U. of penicillin intramuscularly every 3 hours for 18 hours, although the blood sugar was only 90 mg. percent. In another instance, an 87-year-old diabetic male, whose diabetes was well controlled with insulin and diet, received 3 intramuscular daily injections of penicillin in oil and wax, with resultant + + + and + + glycosuria on the 4th day despite occurrence of symptoms of hypoglycemia which were relieved by glucose. The penicillin solution used by Boldyreff (1947) reduced Benedict's solution at a concentration of 10,000 U./cc., which we have confirmed. From the present study, however, it does not appear probable that instances of pseudoglycosuria after massive antibiotic therapy occur except rarely. The purpose of our report is to indicate the possibility that pseudoglycosuria may occur in any individual instance, and to recommend that clinicians be alert for such instances to the end that inappropriate treatment may be avoided.

Summary

A pseudoglycosuria regularly occurs in mice given massive intramuscular injections of Na penicillin G or procaine penicillin G, and occasionally after other antibiotics. Only 2 of

55 patients receiving massive doses of antibiotics showed pseudoglycosuria. Although pseudoglycosuria from this cause may be rare clinically, correct interpretation of this finding is important for proper handling of the individual patient.

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FURTHER INVESTIGATIONS ON THE PHENOMENON OF LEUKERGY*

LUDWIK FLECK

The clumping of white cells in blood, observed in various inflammatory processes, was described by the author as *leukergy* (Fleck, 1947, 1949). In this phenomenon groups of 3 to 20 and more cells are formed with a marked tendency to cytological homogeneity, i.e., some groups contain leukocytes while others consist of lymphocytes or monocytes only.

The technique of the blood test of incubated samples of citrated blood is essentially the same as "tube test" (Fleck, 1949). For this purpose 8 drops of citrated blood are thoroughly mixed with 2 drops of 38% sodium citrate and incubated at 37° C. for 3 hours. A thick blood film is then prepared, dried for 24 hours and stained with diluted methylen blue without fixation. No rinsing is necessary, the stain is removed by tilting the slide. Clumps of white cells are visible under low power on the background of hemolyzed red cells (Figure 1).

Sometimes, particularly in strongly positive leukergy, clumping occurs almost immediately after mixing the blood, but in less pronounced leukergy an incubation period from 1–3 hours is necessary. Incubation for more than 5 hours lessens the intensity of clumping. The degree of leukergy can be expressed by the percentage of clumped white cells of each type (leukergy count). Figure 1 shows the blood of a rabbit 4 days after intravenous injection of 100 million organisms of killed *Escherichia coli*, namely, 34 granulocytes of which 16 are clumped (47%); 56 lymphocytes of which 14 are clumped (25%) and 2 monscytes. Of the total 92 cells 30 are agglomerated (33%). The percentage figures indicate the intensity of leukergy in the examined blood. For reliable results counting of about 1,000 cells for 30–45 minutes is advisable. Before counting the film should be examined under low power to ascertain an even distribution of leukocytes

*From the Academy of Medicine, Lublin, Poland, Department of Microbiology, Director, Professor Ludwik Fleck. Received for publication July 11, 1951.

groups that depends on a thorough mixing of blood (by tilting without stirring).

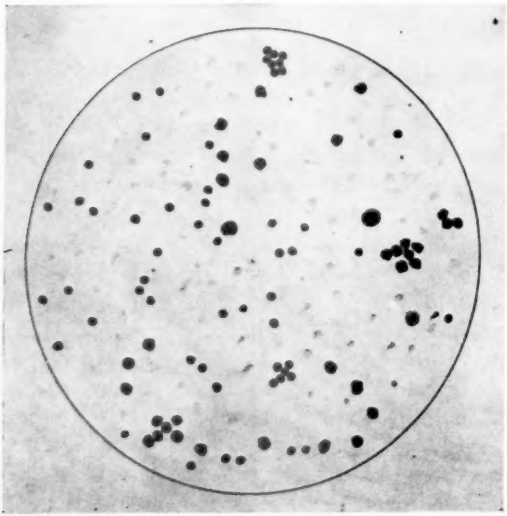

FIG. 1. Leukergy test. Blood smear from a rabbit four days after intravenous injection of one million killed organisms of *Escherichia coli*. 250 X.

In examining blood samples from healthy individuals we have found an average of about 3% of agglomerated cells as compared with 20 to 90% (usually 30–60%) in acute febrile diseases and 10–30% in chronic febrile conditions. Instead of the percentage index the intensity of leukergy can also be evaluated by a scale as follows:

— No agglomeration or less than 5% of cells are clumped.

± Few clumps, generally small (3–5 cells), about 5% of cells are clumped.

+ More numerous and larger clumps, about 10% cells are clumped

+ 20–30% cells agglomerated.

\+ + 30–50% cells agglomerated, average 40%; large groups.

\+ + + 50–60% cells agglomerated, average 55%; large groups.

\+ + + + 70–90% cells agglomerated, average 80%; large groups.

An agglomeration of at least 3 contiguous cells or separated from each other by not more than a cell in diameter is considered a clump.

It has been shown by the author that only inflammatory stimuli can provoke the phenomenon in experimental leukergy. This is easily induced by intravenous injection into a rabbit of killed gramnegative bacteria (Escherichia coli, Salmonella typhi, Proteus vulgaris) in a single dose of at least 50–100 million organisms or by intrapleural introduction of 0.5 or 1.0 ml. turpentine. Less constant is the effect of foreign proteins, peptones or histamine. If foreign proteins are injected into a previously sensitized animal, the effect is more pronounced, but leukergy appears as a rule not before 24–48 hours after injection (St. Borkowska of this institute), whereas injection of bacteria brings it about in 3–5 hours. Large doses of X rays provoking degenerative changes in irradiated organs induce marked leukergy (St. Kordecki of this institute); the same applies to diphtheria toxin which induces leukergy regularly in normal animals, but not in those immunized against a specific agent. The toxins of *B. perfringens* are of lesser effect, the same holds true for the neurotropic toxins of *B. tetanus* and *B. botulinus*.

On the other hand, killed *E. coli* provoke leukergy in immunized animals with a high agglutinin titer just as in normal animals even when the minimal dose of 50 million organisms is used (Szczygielska of this institute). These differences may be explained by the fact that diphtheria toxin neutralizes the toxin completely while the inflammatory action of the bacteria persists. Strong leukergy is caused by the endotoxin of gramnegative bacteria, but the nontoxic hapten obtained from this toxin by hydrolysis is ineffective and so is diphtheria

anatoxin. These results speak for the inflammatory nature of leukergy.

Similarly, the effect of other bacterial toxins and antitoxins can be analyzed. Leukergy is a regular finding in the last five months of normal pregnancy; it increases with the approaching delivery and during labor and lasts about three months after delivery, gradually decreasing (A. Kwiatkowski of this institute). The course of leukergy, leukocytosis and sedimentation rate in pegnancy are not parallel to each other, namely the sedimentation rate after delivery turns to normal earlier than leukergy. Leukergy in pregnancy may be due either to the altered function of the hypophysis and to changes in the vegetative cerebral centers or to the increased activity of the RES. Both interpretations are supplementing each other rather than being contradictory.

Heparin is known to increase the adhesiveness of leukocytes *in vitro* and is a hormone regulating blood circulation produced by "mastzellen" (heparinocytes). These cells scattered around smaller vessels and capillaries form a hormonal system of great physiological importance. Hyperheparinemia found in anaphylactic shocks and after excessive X-ray irradiation is assumed to be responsible for pepton shocks. These facts induced us to make an inquiry into the role of heparin in leukergy.

When heparin is added to blood in the amount of 5–20 mg. per cent, clumping of leukocytes takes place either immediately or approximately 30 minutes after. Microscopic examination reveals small groups of white cells of considerable homogeneity, similar to those usually found in leukergic blood as well as larger groups which contain up to several hundred cells of different types.

The groups of cells in heparinized and in leukergic blood show many similarities: hypertonic solutions (3–4%) inhibit their appearance and cause a disintegration of already formed groups; similar effect is produced by temperatures of 46–48° C., which process is reversible within a limited time; in both instances the clumping proceeds also in the absence of platelets. A distinguishing feature is the inhibition of clumping in heparinized blood by addition of sodium citrate or potassium

oxalate without disintegration of already existing groups. Protamine and toluidine blue do not impede the clumping action of heparin on the leukocytes even if added in amounts exceeding those needed for the neutralization of the anticoagulant.

Intravenous injection of heparin (1.5–2.5 mg/kg body weight) elicits leukergy in rabbits, in some cases within 30 minutes, in others within several hours and persists sometimes for several days, i.e., longer than the anticoagulant action of heparin. No distinction could be detected between leukergy appearing after heparin and leukergy caused by injection of killed bacteria. A preliminary injection of protamine or toluidine blue did not inhibit leukergy caused by heparin or injection of killed bacteria.

Our investigations suggest the conclusion that even if heparin has a share in the mechanism of leukergy increasing the adhesiveness of the white cells, it is by no means the single factor. In anaphylactic shocks and sometimes after X-ray irradiation leukergy appears later than hyperheparinemia and lasts longer than the prolonged coagulation time. In other words, there is no parallel between the anticoagulant action and the degree of leukergy.

Another factor which provokes leukergy is lumbar air insufflation (Stein, 1949). In this case leukergy appears after a few hours, increasing on the following day and lasting for 2–4 days. Leukergy was also found after epileptic seizures and electroshock (Stein, 1949), where it is usually accompanied by fever and leukocytosis. Whether fever and leukocytosis are due to the irritation of diencephalic centers or to meningeal inflammatory reaction, a central regulation of leukergy is feasible.

There is evidence that leukergy occurs in circulating blood. This can be seen in microscopic sections through traumatic extravasations in leukergic animals, where marked clumps of leukocytes can be seen, as compared with scattered leukocytes in hemorrhages of non-leukergic animals. Such clumps can also be seen in bioptic sections of blood vessels of leukergic animals as well as in blood drops immediately after leaving the vessel. Direct microscopic observation of circulating blood in the ear of a leukergic white mouse as well as slit lamp

observations of the conjunctiva of leukergic persons show white clumps of leukocytes and thrombocytes carried with the blood stream. Under favorable circumstances one can also observe in the capillaries of an albino mouse white cells sticking to the vessel's wall as described by Tannenberg, Krogh, *et al.* There can also be seen the formation of leukoytic and thrombocytic clumps rolling along the vessel's wall and occasionally breaking down at the bifurcation of the vessel.

The presence of these cellular agglomerates in the circulating blood in various febrile diseases points to a characteristic stickiness of white cells as their property leading to leukergy. The mechanism of leukergic clumping was studied in a drop of citrated blood from animals deprived of platelets by the use of antithrombocytic serum. Dark field observations showed that clumping results from random collisions of sticky white cells which show fine protoplasmic filaments "synechiae" protruding from their surface and connecting the adjacent cells together. The synechiae, which cannot be seen in slides stained by the ordinary methods, appear not only between homogenous white cells but also connect leukocytes and erythrocytes (Fig. 2, 3). These connections seem to be weaker than between homogenous cells, because leukocytes adhere to leukocytes stronger than to lymphocytes which in turn stick more to lymphocytes than to monocytes or leukocytes. This would explain the cytological homogeneity of cell groups in leukergy without the involvment of serological factors. A similar interpretation was offered by Pijper (1941) in his analysis of the Vi agglutination where the increase and selective adhesiveness is stressed as a cause.

Simultaneously with leukergy provoked in rabbit by injection of about 50 million killed bacteria, a hyperplastic reaction of the bone marrow with granulocytosis and appearance of myelocytes in the blood takes place. In this regard parallel examination of blood and bone marrow of rabbits after bacterial infection are very instructive. Soon after intravenous injection granulocytes disappear from the peripheral blood almost entirely and the white cell count falls to about 2,000 per cub. mm. After 2–3 hours the granulocytes begin to reappear in increased numbers (9,000–10,000 per cub. mm.).

Simultaneously the bone marrow shows an increased leukopoiesis. At this moment leukergy begins. After 5 hours granulocytosis (15,000–18,000) with a shift to the left and a marked leukergy accompanied by a strong hyperplastic reaction of the bone marrow occurs.

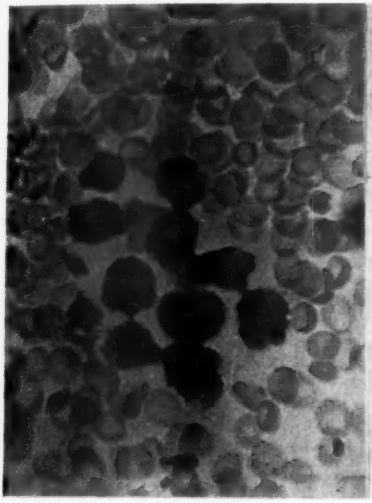

FIG. 2. Leukergy test. Blood smear from a rabbit treated with antiplatelet serum and showing no platelets. Synechiae (protoplasmic bridges) connecting the white cells. 600 X.

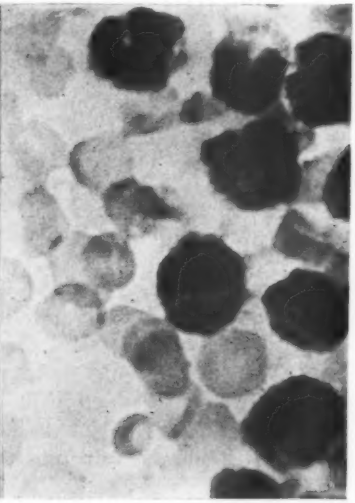

FIG. 3. The same smear as in Fig. 2, showing long radial synechiae connecting adjacent cells. 900 X.

Our observations on leukergy indicate that this phenomenon is the expression of the increased stickiness of granulocytes which are liberated by the bone marrow due to an inflammatory stimulus. These cells do not represent exclusively young elements, since segmented forms always prevail. A positive leukergy test indicating irritation of the bone marrow by an inflammatory process can broadly be compared with the shift to the left, but leukergy is a more expressive symptom manifested by a drastic increase of clumped cells from 3 to 50 and 70 per cent.

It is not established, however, whether leukergy can be entirely explained by the bone marrow irritation alone, since

this would not explain the participation of lymphocytes and monocytes. This would particularly apply to chronic inflammatory states of leukergy where the lymphocytes exceed by far the granulocytes. In this connection Menkin's leukocytosis promoting factor (LPF) and his leukotaxine should be included in the interpretation of the mechanism of leukergy and the possible central regulatory influence.

Attempts of practical application of leukergy test in internal medicine, psychiatry, pediatrics, dermatology and ophthalmology have been initiated. It concerns the detection of subclinical or asymptomatic infections. It is of interest to note that certain apparently healthy rabbits give positive leukergy tests during several days or even weeks showing in their blood from 50 to 80 per cent leukocytes clumped. Systematic investigations in collaboration with F. Stanski and N. Nicewicz have shown in each positive case definite pathological changes never found in rabbits with negative leukergy. Those lesions were in most cases pneumonic foci of different sizes with a serous or sero-fibrinous and cellular exudate into the alveoli with septic congestion and leukocytic and histiocytic infiltration. Microorganisms were not detected in these foci. In other cases the liver showed inflammatory changes in the form of interlobular and perivascular small and round cell infiltration.

Consequently, leukergy offers the most sensitive and reliable test for pathological processes not accompanied by manifest symptoms. It would be therefore advisable to test an experimental animal for leukergy beforehand to eliminate latent infections. This applies particularly to guinea pigs which react in about 80% with positive leukergy without showing pathological symptoms. New-born animals give, as a rule, negative reactions; but when raised with adult animals they change to positive at the age of 4–6 weeks. If the new-born are isolated immediately after birth and weaned artificially, they can be kept leukergy negative, but when a positive guinea pig is added to the cage the test will turn positive after 7–14 days.

The wide range of leukocyte counts in guinea pigs (5 to 21 thousand per cmm) considered as normal is perhaps due to subclinical and abnormal processes caused for instance by

Jackson's salivary gland virus, "L" encephalitis virus, Kurloff bodies, etc. It is therefore possible that our findings point to inapparent infectious processes caused by one or more of these microbes.

The relation of leukergy to other reactions, particularly to sedimentation rate in syphilis and tuberculosis has been investigated by J. Platakis and D. Borecka. These authors found positive leukergy in 13% of 50 patients in various stages of syphilis with a sedimentation rate of 30 mm in 1 hour; thus a relatively high sedimentation rate and weak leukergy. In pulmonary tuberculosis the mean leukergy rate in 167 cases at various stages was 47% and the mean sedimentation rate 40 mm in 1 hour.

In syphilis with purulent skin complications leukergy rises considerably (to 50%) while in tuberculosis with pleural effusion leukergy reaches 60% and sedimentation about 45 mm (mean values). These observations suggest that both sedimentation rate and leukergy vary independently and that the comparison of these data may lead to some considerations as to pathogenesis.

In cases of syphilis treated with penicillin and mapharsen, leukergy rises in the first few days up to 40–50% and decreases rapidly in spite of further treatment to the initial values. Since neither penicillin nor mapharsen cause leukergy in healthy individuals, the increased stickiness of leukocytes could likely be explained by the inflammatory action of the derivatives from destroyed treponemas and related to Herxheimer's reaction. Perhaps a similar phenomenon will be found in other diseases leading to evaluation of the therapeutic effect or to strain resistance.

When tuberculin is injected even in large quantities intradermally to human or animals, no leukergy was observed despite of tuberculous allergy; but in patients with active tuberculosis the injection causes leukergy or raises it to higher titers. In rabbits infected with the virulent strain of bovine tuberculosis, intradermal injection of tuberculin (1.2 ml 1:100) causes leukergy beginning with the second week after infection and during the entire course of illness until death. The leukergy peak is reached in 48–72 hours after injection. In

rabbits infected with human strain of low virulence such reaction is possible only during the first week after infection. Once the process has been arrested and the abscess encapsulated, leukergy will not be induced by tuberculin, but with recrudescence of the process (dissemination) leukergy can be provoked. Rabbits infected with B.C.G. strain usually show an increase of leukergy following tuberculin during the first 6 weeks after which time leukergy cannot be provoked even if the positive skin test persists. Provocation tests were applied to patients with *lupus vulgaris* with the result that in 14 cases tested the leukergy index rose from 20% to 45% within 48–72 hours falling afterwards to initial values. Thirty additional cases of *lupus vulgaris* confirmed these findings, whereas in ten control cases (lupus erythematosus, acne vulgaris) no change in the leukergy index was found.

The effect of tuberculin on leukergy can be attributed to focal reaction which in inactive tuberculosis is missing. On the contrary, active tuberculosis responds to tuberculin injection with a subclinical focal reaction which can be detected by leukergy test. Similar investigations on brucellosis are in progress.

Summary and Discussion

Leukergy is a component of inflammation manifested by the appearance in the peripheral blood of groups of white cells, mainly granulocytes, and characterized by an apparent stickiness of their surface. It would seem that the granulocytosis is due to a hyperplastic stimulation of the bone marrow and to a sudden liberation of granulocytes following an inflammatory stimulus.

Leukergy is a very common symptom which can be elicited by various extrinsic stimuli, such as pathogenic organisms or killed bacteria or bacterial toxins, inflammatory processes, and by foreign proteins especially in sensitized animals. Leukergy is as common as fever, leukocytosis or increased sedimentation rate, but it is not congruent with any of them, and can appear without any of them. It can appear without fever, with leukopenia and a low sedimentation rate. Leukergy is a very sensitive symptom, much more so than any of the above mentioned,

and therefore allows the detection of latent infections or subclinical inflammatory conditions.

The correlation of leukergy with other inflammatory symptoms can lead to such important conclusions as differentiation of the high sedimentation rate of cachectic patients from that of inflammatory conditions, as well as the separation of the physiological leukocytosis from an inflammatory. Furthermore, it allows to detect an inflammatory action of a toxin, or of bacterial cells destroyed by antibiotics and to study the processes of infectious diseases or of an anaphylactic shock. In allergic states leukergy can furnish a specific test. Injection of tuberculin causes in some tuberculous patients an increase of leukergy while in others not, independently of the intensity of skin reaction. An increase in leukergy due to tuberculin response points to the presence of an active tuberculous process. Leukergy index can prove helpful in hematological examinations being more sensitive than the shift to the left. Leukergy can be considered as an important special function of leukocytes and is probably regulated by the central nervous system which influences the migration of leukocytes and phagocytosis.

Conclusions

Only inflammatory factors can provoke leukergy.

Leukergy accompanying normal pregnancy can be due either to increased inflammatory readiness or to changes in the CNS, namely in the hypophysis and vegetative centers. In this connection lumbar air insufflation should be considered.

The presence of clumped leukocytes in circulating blood has been observed.

The mechanism of leukergic clumping of leukocytes consists in random collisions of the sticky cells and most probably not in their attraction at distance. The adhesiveness of cells of the same type is stronger than that of different types with the result of homogeneity of agglomerations.

Hyperplastic bone marrow irritation can be found during leukergy. Parallel examination of blood films suggests that the leukergic cells have escaped from the bone marrow due to an inflammatory stimulus.

The use of leukergy as a test for detection of asymptomatic infections is described.

The provocation of leukergy by tuberculin is suggested as a test for active tuberculosis.

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HAZARDS OF VAPORS OF CHLORINATED AND OTHER HYDROCARBONS IN CONFINED SPACES*

LAURO GUTIERREZ-VELA

Exposure of workmen to chemical mists and vapors or to harmful gases and dusts is increasingly important. The relatively new chemicals used in the control of insects and rodents in grain storages and ships' holds have made necessary a quick method for analysis of chlorinated hydrocarbons in connection with the loading of grain in ships. It is imperative, just as it is in petroleum carriers, that a grain cargo be trimmed as soon as possible in order that the loading be completed and the ship allowed to sail with the least delay. Shipments of grain from the Port of Galveston were 99,764,938 bu. in 1947, 107,536,647 bu. in 1948, 110,218,041 bu. in 1949, and 80,710,361 bu. in 1950.

The chlorinated hydrocarbons are harmful in varying degrees not only because of their inherent properties, as with many other organic solvents, but also because of their thermal decomposition with the formation of phosgene, hydrogen chloride, and chlorine. The order of toxicity of the more common products with which we have dealt range from one part per million for phosgene to 100 p.p.m. for trichloroethylene. Other toxic compounds as chloropicrin, carbon bisulfide, carbon tetrachloride, methyl bromide, benzene, sulfur dioxide, methyl chloride, and dichloroethylene have been encountered. In general, these compounds appear in mixtures, which contain one or more of the chlorinated hydrocarbons. These mixtures are dissolved or dispersed in a suitable solvent, which itself may cause injurious or toxic effects.

Values for maximum allowable concentrations (M.A.C.) have been based principally on recommended allowable concentrations for daily eight-hour exposures as given in the report submitted by the Committee of Threshold Limits, American Conference of Governmental Industrial Hygienists, in their

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1947 and 1948 meetings. In mixtures of several hazardous agents, M.A.C. has been based on the most toxic compound. CCl_4, which has a M.A.C. of 50 p.p.m., is usually the determining constituent, but some of these compounds may be synergistic and the effect of mixtures on living tissue may be unpredictable without further pharmacologic research.

Methods

The quantitative analysis of chlorinated hydrocarbons in air is not new and has been dealt with in various chemical and toxicological works. The following simple method has been used for the estimation of chlorinated hydrocarbon vapors in ships' holds and other chemically treated compartments. A sample of the air is introduced into a slow-combustion pipette which contains a few ml. of water. The gas is decomposed by burning for a few minutes, the heat required being generated by an electric current passing through a calibrated platinum wire similar to the combustible gas indicator filament. The temperature of the reactor is not critical; 400 to 500° C. will decompose most of the chlorinated hydrocarbons in 5 to 10 minutes.

The hydrogen and chloride ions formed are dissolved in the water present in the tube and the acid formed is titrated by any of several available methods. A weak solution of sodium arsenite is satisfactory. An alcoholic solution of bromcresol green and methyl red is used as an indicator.

This method is adequate for halogenated hydrocarbons, and in particular for chlorinated hydrocarbons. This procedure for decomposing the sample and recovering the halogen in a form suitable for estimation by any standard method offers the distinct advantages for its present applications: short time required for analysis; simplicity of apparatus without sacrifice of accuracy and precision; and the ease with which stable compounds such as chlorinated hydrocarbons are decomposed.

Carbon Bisulfide

Carbon bisulfide is another compound frequently used as a fumigant, especially in mixtures. The combustible gas indicator can be used for its detection; however, only relatively

high concentrations are indicated, and these are on the low side unless calibration curves are available. The M.A.C. for this substance is only 20 p.p.m., which immediately demands a sensitive method for its detection and quantitative estimation.

The modified diethylamine method of Viles is satisfactory. The reagent consists of a mixture of ethyl alcohol, diethylamine, triethanolamine, and a weak solution of copper acetate. A sample of air is drawn through a bubbler unit, containing glass beads, and connected to the air intake of an aspirator. Two or three ml. of the reagent are placed in the bubbler trap. Water is then allowed to flow out of the aspirator bottle and the air flow regulated so that 200–300 ml. air sample is collected in about 15 minutes. The contents of the bubbler trap are emptied into a beaker and the bubbler and beads washed with a few ml. of the reagent. This is transferred to a 50 ml. Nessler tube and the volume made up to 10 ml. A light amber color develops in a few minutes. The intensity of the color indicates the concentration of the gas by comparison with color standards which can be used to measure accurately concentrations of 2 p.p.m. of CS_2.

Since the chief interest is the detection rather than quantitative estimation of this compound, we use a modification of the Viles method in which the air is passed through a U-tube containing the reagent, and if found positive the use of a suitable breathing apparatus is recommended.

Discussion

While it may be considered that the complex pharmacological action of a toxic substance on living organisms cannot really follow the methods of physical chemistry for profitable mathematical analysis, investigation of the toxic agent just prior to its absorption by a human being can be made to show how a single variable changes the situation considerably.

The equilibrium between a liquid and its vapor is dependent only on the temperature and pressure. The vapor pressure of a liquid is a constant quantity at any temperature, and is independent of the amount of liquid and vapor present, but it increases as the temperature raised. The rate of change in vapor pressure is given by Clapeyron's differential equation:

$$\frac{dp}{dt} = \frac{l_v}{T(v_2 - v_1)},$$

where T = absolute temp.; l_v = heat of vaporization; v_1 = specific vol. of liquid; and v_2 = specific vol. of vapor.

This equation can be used to calculate the rate of change in v.p. of these toxic compounds when the temperature approaches the b.p. Thus, for CS_2

$$\frac{dp}{dt} = \frac{(352)(0.04129)}{(319.3)(0.345-0.001)} = .132 \text{ at m./degree}$$

and $760 \times .132 = 100$ mm/degree, since $l_v = 352$ cal./g.; $T_{B.P.} = 319.3°$ K; $v_1 = 0.833 = 0.0008$ l.; $v_2 = 345 = 0.345$ l.; and 1 cal. = 0.04129 l. atm.

Higher temperatures therefore lead to a higher incidence of toxic effects due to the increased volatility of the material with corresponding increased concentration. In practice, the majority of cases of illness due to air borne contaminants occur in the summer months when the temperature in a ship's hold may be as high as 100° F. Control of a single variable, the temperature, can reduce the toxic concentration of these hazardous agents to such a degree that it may substantially reduce, or even eliminate, the harmful effects of these materials in confined spaces.

Summary

Simple methods are noted for rapid detection and estimation of concentrations of chlorinated hydrocarbons and carbon bisulfide well below their maximum allowable concentrations, and the practical importance of the relation between temperature and vapor pressure is discussed.

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THE RELATIONSHIP OF SODIUM INTAKE TO THE HYPERTENSIVE HYALINOSIS SYNDROME PRODUCED IN THE RAT BY PARABIOSIS*

I. Hypertensive Cardiovascular Disease

C. E. Hall and O. Hall

When rats are chronically treated with excessive amounts of desoxycorticosterone acetate (DCA), they develop a syndrome which consists of polydipsia, polyuria, hypertension, nephrosclerosis, periarteritis nodosa and myocardial lesions reminiscent of those observed in rheumatic heart disease (Selye and Hall; Friedman *et al.*). In many, if not all cases, a type of mono- or poly-arthritis is an associated manifestation, although it usually precedes temporally the development of hypertensive cardiovascular disease (Selye, Sylvester, Hall, and Leblond, 1944; Pirozynski and Akert, 1949). Selye (1950) refers to these conditions, which are characterized by the deposition of an amorphous, eosinophilic hyalin material in and around blood vessels and connective tissues, as "hyalin diseases."

Evidence has been adduced that at least the hypertensive cardiovascular manifestations are dependent upon sodium, since additional sodium intake aggravates them, while diets in which the sodium is so low as to be negligible prevent the development of hypertensive cardiovascular disease in DCA-treated rats (Selye, 1949). Recently we have shown that when female rats are placed in parabiosis and given 1% sodium chloride solution to drink, a malignant hypertension with concomitant nephrosclerosis, periarteritis nodosa and severe myocardial lesions—a syndrome indistinguishable from that produced by overdosage with desoxycorticosterone—spontaneously develops in about 40% of the pairs (Hall and O. Hall). Since there remained the remote possibility that the hypertensive syndrome was caused by an unusual susceptibility of parabiotic rats to sodium chloride, the present experiments designed to test this contingency were undertaken.

*From the Carter Physiology Laboratory, The University of Texas Medical Branch, Galveston. This study was supported by a grant from the American Heart Association. Received for publication August 8, 1951.

In order to stress the similarity of the syndrome provoked by parabiosis to that produced by DCA it should be mentioned that arthritis has commonly been observed to occur in parabiotic rats. Since the joint involvement is independent of the hypertensive condition while the cardiovascular lesions are not, arthritic manifestations are to be described in a subsequent communication.

Experimental

Some one hundred and fifty litter-mate female rats from our colony, the parent stock of which was originally obtained from the Holtzman-Rolfsmeyer Co., weighing between 65 and 85 gms and paired to within 5 gms for the operation, were placed in parabiosis according to the method of Bunster and Meyer (1933). It has been found preferable, however, to employ interrupted cotton thread sutures rather than the metal wound clips which are commonly used to join the skin flaps; and No. 25 linen thread is considered preferable to catgut for scapular sutures.

Half of the animals, group 1, were allowed to drink tap water (found by flame photometer analysis of several samples to contain 31.5–41.5 mg per cent sodium); while the other half, group 2, were given distilled water (sodium content undetectable). All animals received Purina Laboratory Chow (sodium content *circa* 0.97%) *ad libitum*. About 30% of the animals in both series developed evidences of "parabiotic intoxication" (Finerty and Panos, 1951), and either succumbed or were discarded from the experiment because of evident impairment of both growth and health of one of the partners. A further 15% developed infections or infected adhesions and were also discarded. Such infections are almost invariably delayed in onset and seem to reflect an incompatibility between tissues rather than a primary infection at operation. For about two weeks after parabiosis in such cases the incisions show perfect healing and no signs of infection can be detected. Suddenly thereafter there occurs an extensive tissue necrosis accompanied by a suppurative infection.

Nineteen pairs in group 1 and nineteen pairs in group 2 survived in excellent condition for three weeks or longer and

were sacrificed at periodic intervals thereafter. Blood pressures were taken once weekly on each of the rats beginning one week after the operation. The tail plethysmograph method of Williams, Harrison, and Grollman (1939) as modified by Sobin (1946) was used for the blood pressure determinations and the rats were unanaesthetized. In each instance the arithmetic mean of four consecutive determinations, agreeing to within 5 mm. Hg, was taken as representative of the blood pressure. Although we do not normally encounter pressures in excess of 135 mm. Hg in normotensive rats, we have arbitrarily designated 150 mm. Hg as being the lowest pressure consonant with a diagnosis of hypertension in the rat. At autopsy the animals were carefully separated and weighed; the kidneys, adrenal glands, cardiac ventricles, thymus and portions of the mesentery and pancreas were excised for weight and/or histology. Bouin's fluid was used as the fixative and all organs were weighed after fixation.

Experimental Results

The rats receiving tap water behaved essentially similarly to those given 1% NaCl to drink and reported previously (Hall and Hall, 1951). Beginning as early as the 10th day postoperatively, more commonly some time in the second or third postoperative week—and occasionally even in the fourth week—hypertension began to develop in one of the partners in eight of the nineteen pairs. The attached parabionts did not develop hypertension, but in almost every case showed a more or less marked hypotension (blood pressure 60–85 mm. Hg) for one or two weeks prior to, or occasionally along with, the hypertension which developed in their partners. So consistent was this occurrence that it was possible to predict the development of hypertension in a rat if the attached parabiont showed a marked hypotensive episode of more than one week's duration. Since the blood pressure determinations were made only once weekly, it is possible that a hypotensive episode in one of the partners preceded the onset of hypertension of the other in every case, but this could easily have been missed. Both partners in the remaining eleven pairs remained normotensive.

The incidence and severity of the hypertension observed in the animals receiving tap water as the drinking fluid was indistinguishable from that reported previously to occur in parabiotic rats given 1% sodium chloride to drink. Unlike the sodium chloride-treated rats, however, none of this or the succeeding group ever exhibited any sign of edema or anasarca.

The blood pressure curves on normotensive-normotensive and hypertensive-normotensive pairs in the tap water treated group are given in Figs. 1A and 1B, and the body and organ

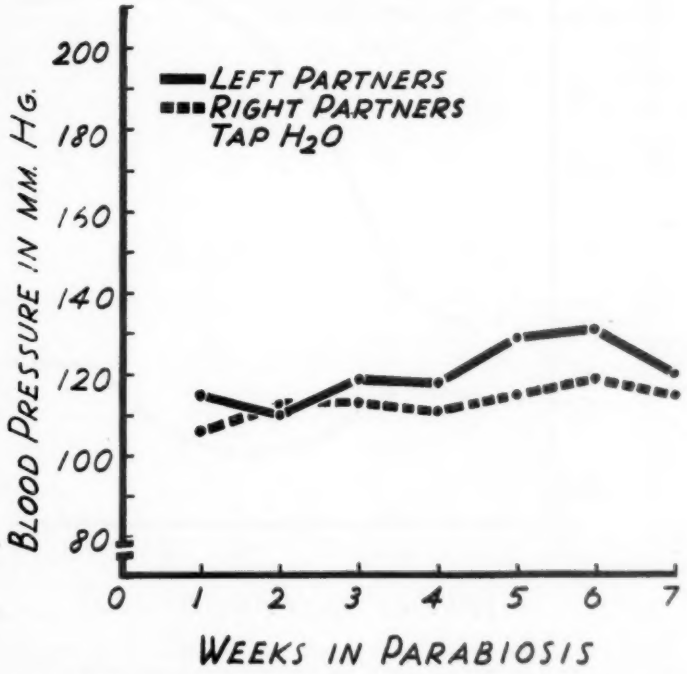

FIG. 1A. Average blood pressure in left and right partners in eleven pairs of normotensive parabiotic rats receiving tap water to drink.

weights with their standard errors, together with the incidence and severity of cardiovascular lesions, is given in Table I.

A perusal of Table 1 indicates that hypertensive animals develop a marked cardiac and renal hypertrophy. The adrenal weight of hypertensive animals also tends to be greater than that of normotensive rats. Periarteritis nodosa of the mesenteric, splenic and pancreatic arteries was grossly evident at autopsy in two of the eight hypertensive rats (Figs. 2, 3, and 4), and microscopically visible in five of them.

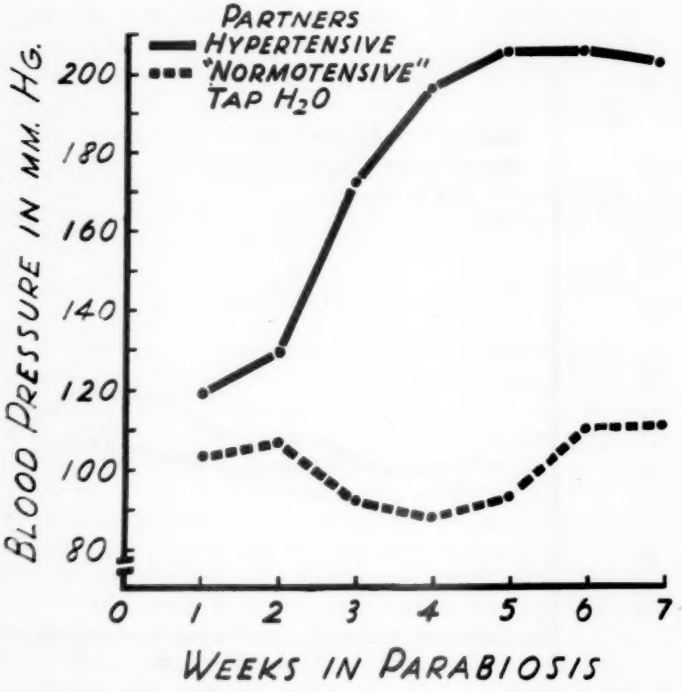

FIG. 1B. Average blood pressure in eight pairs of parabiotic rats, receiving tap water to drink, in which one of the partners became hypertensive. Note the tendency to hypotension in the attached parabiont.

In the animals of group 2, receiving distilled water to drink, hypertension—again usually preceded by an hypotensive episode in the attached parabiont—developed in seven of the

nineteen pairs, the other twelve remaining normotensive. The blood pressure curves of the pairs is given in Figs. 5A and 5B. The elimination of sodium chloride from the drinking fluid did not seem to alter significantly the overall incidence of hypertension, however the course of the disease was different. In three of the seven hypertensive animals—whose blood pressure had reached 175, 196, and 199 mm. Hg on the 21st, 29th, and 29th day, respectively—the blood pressure had declined to normal levels of 118, 134, and 126 mm. Hg, respectively, by the 42nd, 42nd, and 35th day of parabiosis, at which time the animals were sacrificed. Of these three animals only the last, whose blood pressure following the onset of hypertension had declined to normotensive levels only during the week prior to sacrifice, showed the presence of renal and cardiac lesions. This tendency of the blood pressure to rise rapidly to hypertensive levels, and then decline to normal

TABLE I

Body and Organ Weights Together with the Incidence and Severity of Cardiovascular Lesions in Normotensive and Hypertensive Parabiotic Rats on Tap Water‡

DATA		GROUP			
		Normotensive + Normotensive Left Partners (11)	Normotensive Right Partners (11)	Hypertensive + Normotensive Hypertensive Partners (8)	Normotensive Normotensive Partners (8)
Body Weight (gm)	Initial	83 ± 4	83 ± 4	74 ± 3	76 ± 3
	Final	156 ± 8	154 ± 6	137 ± 12	129 ± 16
Heart Weight	in gm.	.437 ± .018	.446 ± .013	.632 ± .036	.380 ± .023
	% B.W.*	.281 ± .009	.291 ± .012	.444 ± .029	.317 ± .028
Kidney Weight	in gm.	1.253 ± .055	1.302 ± .051	1.589 ± .097	1.219 ± .090
	% B.W.*	.803 ± .071	.849 ± .033	1.232 ± .140	1.015 ± .100
Adrenal Weight (mgm)		53.1 ± 2.8	55.0 ± 2.7	59.8 ± 3.1	52.4 ± 1.6
Thymus Weight (gm)		.279 ± .018	.251 ± .011	.180 ± .052	.188 ± .045
Vascular Lesions					
Heart	Incidence	0	0	5	0
	Severity	0	0	+ +	0
Kidney	Incidence	0	0	5	0
	Severity	0	0	+	0
Periarteritis†	Incidence	0	0	5	0
	Severity	0	0	+ +	0

*B.W. = Final body weight.
†= Pancreas and mesentery.
‡Na content approximately 31.5–41.5 mg percent.

levels, has only been observed to occur in parabiotic pairs receiving distilled water.

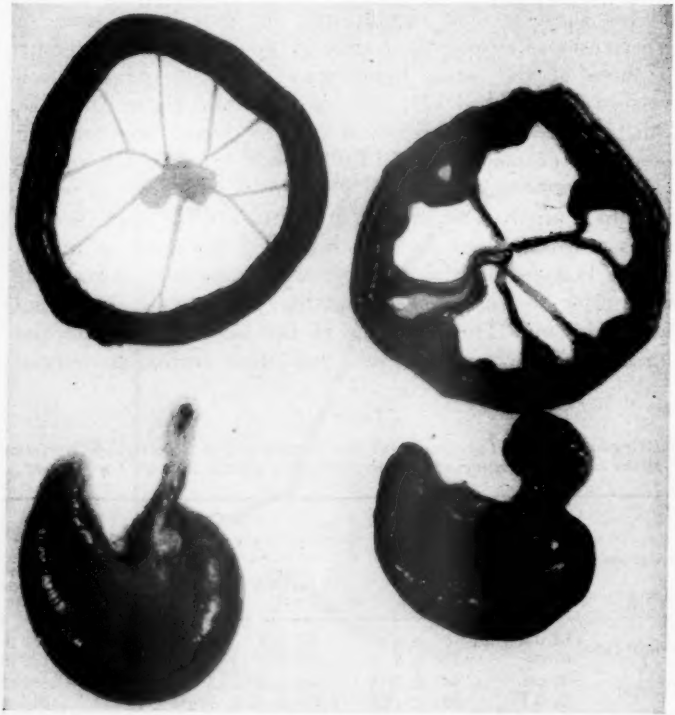

FIG. 2. Duodenal loops with attached mesenteries and the caeca from a pair of parabiotic rats united for 35 days in which one partner had been hypertensive for at least three weeks. Note the presence of periarteric nodules in the branches of the mesenteric arteries in the hypertensive animal on the right and the normal appearance in the normal animal on the left. The caecum from the normotensive rat is normal in appearance; whereas that from the hypertensive rat is intensely inflamed. Severe periarteritic lesions were present in the latter.

In contrast to the groups treated with 1% NaCl and tap water, respectively, none of the animals receiving distilled water were found at autopsy to have gross lesions of periarteritis nodosa, nor were such lesions detectable microscopically in either the

mesenteric, splenic, or pancreatic arteries. Similarly nephrosclerosis and cardiac lesions, frequently detectable at autopsy in hypertensive parabiotics on either 1% NaCl or tap water, were observed only on histologic examination in those receiving distilled water. Periarteritic involvement of the myocardium was also observed microscopically in some of the latter group of hypertensive rats. The body and organ weights and their standard errors, together with the incidence and severity of cardiovascular lesions are given in Table II. It will be observed that here, as in the case of the previous group, cardiac and renal hypertrophy were both manifest in the hypertensive rats. The adrenal glands were only slightly larger in hypertensive than in normotensive rats.

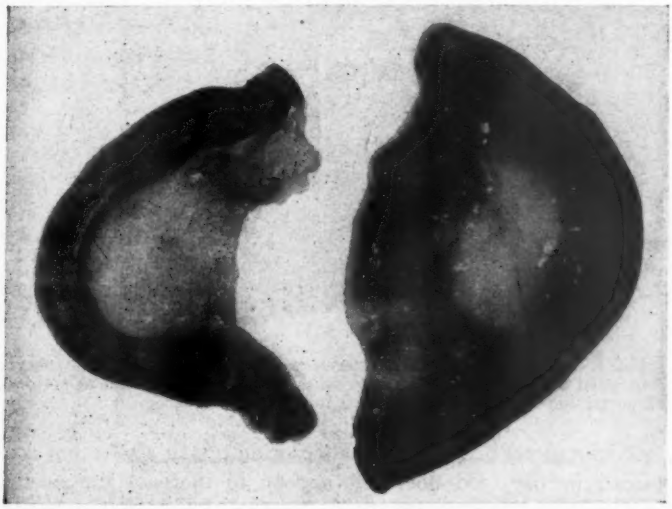

FIG. 3. Periarteritis nodosa in the pancreas from a hypertensive rat (right) contrasted with the normal appearance of the tissue from the normotensive parabiont (left).

Discussion

These studies support the observation made previously that hypertensive cardiovascular disease develops in one of the partners in approximately 40% of parabiotic pairs of female

rats; and that hypotension often develops transiently in the attached parabiont in such cases. It would also appear that the hypertension spontaneously occurring in parabiotic rats is not dependent upon an unusually high NaCl intake.

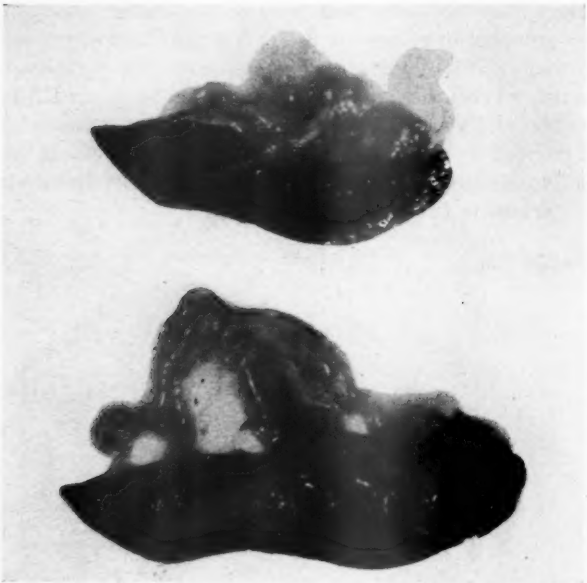

FIG. 4. Periarteritis in splenic arteries of a hypertensive parabiont (above) contrasted with the normal structure in the normotensive parabiont (below). Hypertension for at least three weeks in the former.

While increasing the sodium chloride intake above that contained in the food does not appear to increase the overall incidence of hypertension, it does appear to prevent the hypertension once developed from regressing. The severity of the concomitant cardiovascular lesions is also diminished in hypertensive rats by lowering the NaCl intake. Within the time limits of our studies, the development of periarteritis nodosa in the splenic, pancreatic, and mesenteric arteries in hypertensive rats appears to be completely dependent upon increasing the NaCl intake above that normally contained in the diet.

It is possible of course that such lesions would have developed had the period of observation been extended. No attempt at evaluating the effect of low sodium diets has been made. Distilled water was employed simply because the tap water available to us is excessively high in sodium content, and we merely wished to determine whether excessive sodium intake was essential to the development of parabiotic hypertension, or whether it was possible to elicit the syndrome by employing a normal diet.

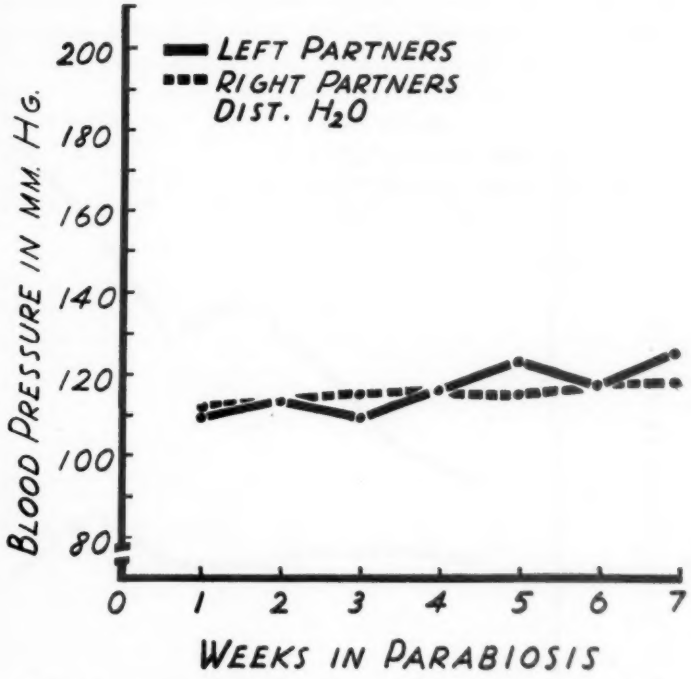

FIG. 5A. Average blood pressures in left and right partners in twelve pairs of normotensive parabiotic rats receiving distilled water to drink.

The development of hypertension, cardiac and renal hypertrophy in parabiotic rats, together with the close correspondence of the lesions to those produced in the rat by DCA, suggests

a common pathogenesis. It is tempting to consider the syndrome as being caused by adrenal hyperactivity characterized by a relative preponderance of mineralocorticoids over glucocorticoids. This is suggested by the augmenting effect of sodium on the incidence and severity of the lesions and by the fact that the adrenal glands tend to be larger in hypertensive animals. If the enlarged adrenals were producing an excessive glucocorticoid secretion, then one would expect that the cardiovascular lesions would be minimized or prevented (Woodbury, Rosenberg, and Sayers, 1950; Friedman, Friedman, and Nakashima, 1950) and that a greater degree of thymic atrophy would be observed.

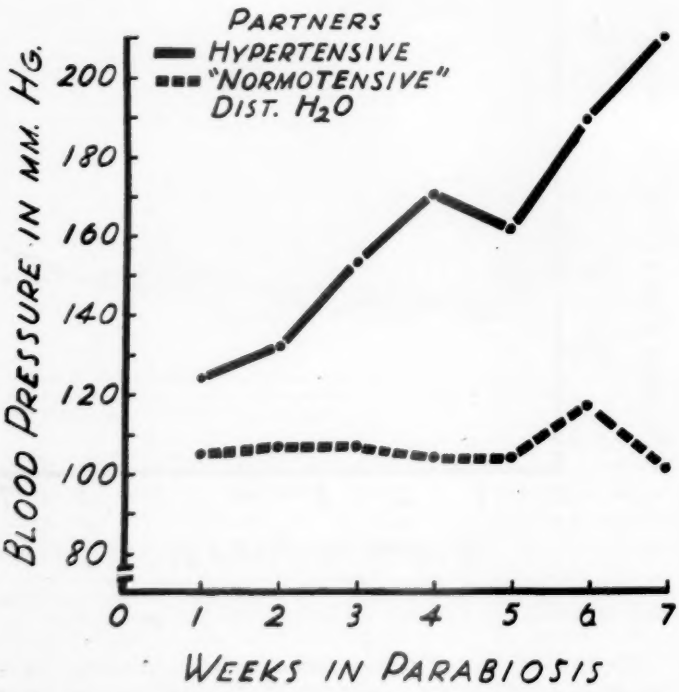

FIG. 5B. Average blood pressures in seven pairs of parabiotic rats in which one of the partners developed hypertension.

It seems possible that the condition of parabiosis imposes a sufficient stress in a rather constant (i.e., about 40%), number of animals, to cause one of the pair to become hypertensive. This may be augmented or conditioned by an anaphylactic type of response to foreign blood proteins crossing the capillary anastomosis. In a total of over 200 pairs of parabiotics studied by us in these and other experiments however, hypertension has never been encountered in both members of a pair. The reason for this is not apparent, but this dissimilarity of blood pressure in parabiotics is not confined to rats, since it has also been reported in human parabiotic pyopagus twins (Jones, Younghusband, and Evans, 1948). The cardiovascular lesions appear to be strictly dependent upon the hypertensive state, whereas lesions in normotensive animals have never been obsrved by us, regardless of whether these were in parabiosis with hypertensive or other normotensive animals, typical periarteritic lesions in various vascular territories as well as

TABLE II

Body and Organ Weights Together with the Incidence and Severity of Cardiovascular Lesions in Normotensive and Hypertensive Parabiotic Rats on Distilled Water

DATA		GROUP			
		Normotensive + Normotensive Left Partners (12)	Normotensive Right Partners (12)	Hypertensive + Hypertensive Partners (7)	Normotensive Normotensive Partners (7)
Body Weight (gm)	Initial	76 ± 2	77 ± 2	80 ± 6	82 ± 6
	Final	156 ± 6	150 ± 5	164 ± 10	139 ± 13
Heart Weight	in gm.	.535 ± .020	.474 ± .015	.738 ± .059	.491 ± .037
	% B.W.*	.356 ± .015	.317 ± .005	.449 ± .027	.363 ± .031
Kidney Weight	in gm.	1.408 ± .047	1.403 ± .033	1.780 ± .077	1.366 ± .083
	% B.W.*	.907 ± .024	.941 ± .021	1.093 ± .045	1.009 ± .070
Adrenal Weight (mgm)		56.7 ± 4.0	51.6 ± 3.7	59.6 ± 3.4	53.2 ± 3.4
Thymus Weight (gm)		.273 ± .013	.240 ± .022	.240 ± .014	.222 ± .036
Vascular Lesions					
Heart	Incidence	0	0	4	0
	Severity	0	0	+ +	0
Kidney	Incidence	0	0	4	0
	Severity	0	0	+	0
Periarteritis†	Incidence	0	0	0	0
	Severity	0	0	0	0

*B.W. = Final body weight.
† = Pancreas and mesentery.

nephrosclerosis have been observed in many animals—not reported in this series—which have been hypertensive for only a few days.

Summary

When female rats are placed in parabiosis, hypertension develops in one of the partners in about 40% of the pairs.

If tap water (31.5–41.5 mg. % NaCl) is given as the drinking fluid the elevated blood pressure is progressive and usually leads to the development of severe and widespread cardiovascular lesions. The substitution of distilled water does not affect the incidence of hypertension, but the elevated blood pressure tends to return to normal levels in many cases.

Renal, adrenal, and cardiac hypertrophy, accompanied by nephrosclerosis, islets of hyalinization in the myocardium, and widespread panarteritis of the type seen in periarteritis nodosa, are characteristic of the hypertensive animal. The reduction in dietary sodium which results from the substitution of distilled water for tap water prevented, in short term experiments, the development of panarteritis in the pancreatic, splenic, and mesenteric arteries. Nephrosclerosis and myocardial lesions were not so prevented, but seemed to be of lesser severity.

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THE RELATIONSHIP OF SODIUM INTAKE TO THE HYPERTENSIVE HYALINOSIS SYNDROME PRODUCED IN THE RAT BY PARABIOSIS*

II. Arthritis

O. Hall and C. E. Hall

In a series of previous communications it has been reported that when female rats are placed in parabiosis, one of the partners develops malignant hypertension in about 40% of the pairs. This is accompanied by cardiac, adrenal, and renal hypertrophy and by nephrosclerosis and widespread panarteritis or periarteritis nodosa (Hall and Hall). The syndrome is strikingly similar to that which is observed to occur in rats treated with excessive amounts of desoxycorticosterone acetate (DCA) (Selye and Hall, 1944). Just as in the case of desoxycorticosterone treated rats, sodium restriction modifies the course of hypertension and the vascular lesions consequent to the elevated blood pressure (Hall and Hall, 1951). This suggests that the syndrome of parabiotic hypertension may be one with which the adrenal cortex is intimately concerned. A possibility which is emphasized by the adrenal enlargement so commonly encountered in hypertensive parabiotic rats.

Rats treated with excessive amounts of DCA also frequently develop arthritis (Selye; Pirozynski) and it is the purpose of the present communication to report the occurrence of arthritis in parabiotic rats.

Materials and Methods

Approximately two hundred litter-mate pairs of female rats from our colony, the parent stock of which was originally obtained from the Holtzman-Rolfsmeyer Company, weighing between 55 and 100 gms, paired to within 5 gms for the operation, were placed in parabiosis according to the technique of Bunster and Meyer with the modifications previously described. These were divided into three groups. Group 1 was

*From the Carter Physiology Laboratory, The University of Texas Medical Branch, Galveston. This study was supported by a grant from the American Heart Association. Received for publication July 16, 1951.

allowed to drink a 1% NaCl solution; group 2 was given tap water (several samples of which yielded sodium values of 31.5–41.5 mg % by flame photometer analysis); and group 3 received distilled water in which sodium could not be detected. All animals received Purina Laboratory Chow *ad lib.* (sodium content *circa* 0.97%). Many of the animals either succumbed to parabiotic intoxication or were discarded from the experiment because of an obviously impaired physical condition. Only animals which maintained a satisfactory state of health, as judged by general appearance and growth, were retained. Many of the rats in each group developed malignant hypertension, which has been reported in previous communications, and these were sacrificed along with normotensive controls at periodic intervals as described earlier (Hall and Hall, 1951c).

Daily examinations of the pairs were made in order to ascertain their condition and, since articular swellings were observed in some of the parabionts, the appearance of the foot and ankle joints was recorded each day.

Results

During the course of routine examination it was noted that many of the animals in each of the groups developed swollen joints. The tarso-metatarsal joints were most frequently involved although the metacarpals did not entirely escape. The feet were not usually swollen, turgid, and inflamed as is characteristically the case in the so-called "topical irritation arthritis" and the infectious arthritides occasionally encountered in rats with pyogenic infections due to a rat bite or laceration. Rather the lesion typically appeared as a sharply circumscribed, nodular swelling of the metatarsal or ankle joint region; although sometimes adjacent swellings tended to coalesce thus producing a rather uniform swelling of the entire foot. As in the case of arthritis produced by treatment with DCA, the lesion in this case tended to flit from joint to joint and to appear and disappear in an irregular and unpredictable fashion. Occasionally it developed in both members of a partnership, but seldom synchronously. In a few cases it developed into a chronic arthritis lasting for 2–3 weeks. Such joint manifestations have been noted as early as twelve days

and as late as twenty-nine days postoperatively; however seventy-five percent of the rats which developed arthritis did so between the twelfth and twenty-first day.

Insofar as the overall incidence is concerned there was no difference apparent between the three groups: nor did there seem to be any predilection for hypertensive animals as compared with normotensives. The lesion, both grossly and microscopically, resembled that produced by DCA.

The hypertension, which has been reported previously, is invariably confined to one of the pair of parabionts. For this reason the pairs are designated as normotensive-normotensive or hypertensive-normotensive partnerships. The overall incidence of arthritis in both normotensive and hypertensive animals of the three groups is given in Table I. The gross appear-

TABLE I

Incidence of Arthritis in Normotensive + Normotensive and Hypertensive + Normotensive Pairs of Parabiotic Rats on Different Sodium Intakes

Data	Normotensive + Normotensive Partner		Hypertensive + Normotensive Partner	
	Left	Right	Left	Right
Group 1 (1% NaCl)				
Total Number	15	15	12	12
Incidence of arthritis.	6	1	2	1
Group 2 (Tap H_2O				
Total Number	16	16	9	9
Incidence of arthritis.	3	2	4*	2
Group 3 (Distilled H_2O)				
Total Number	12	12	7	7
Incidence of arthritis.	2*	2	4*	2

*Indicates that one pair, in each of these groups, had arthritis in both members of the partnership.

ance of the lesion as it is manifest in the hind foot is depicted in Figs. 1 and 2: while Figs. 3–7 illustrate the histopathologic transformations in the structure of the joints and adjacent tissues. The most common changes which were encountered consisted of marked edema of the synovial membranes with concomitant hydrarthrosis. Frequently such changes progressed to disintegration of the membrane with pronounced hyalin necrosis of this and adjacent tissues. Fibrinoid necrosis of the tendon sheaths and subcutaneous tissues of the periarticular

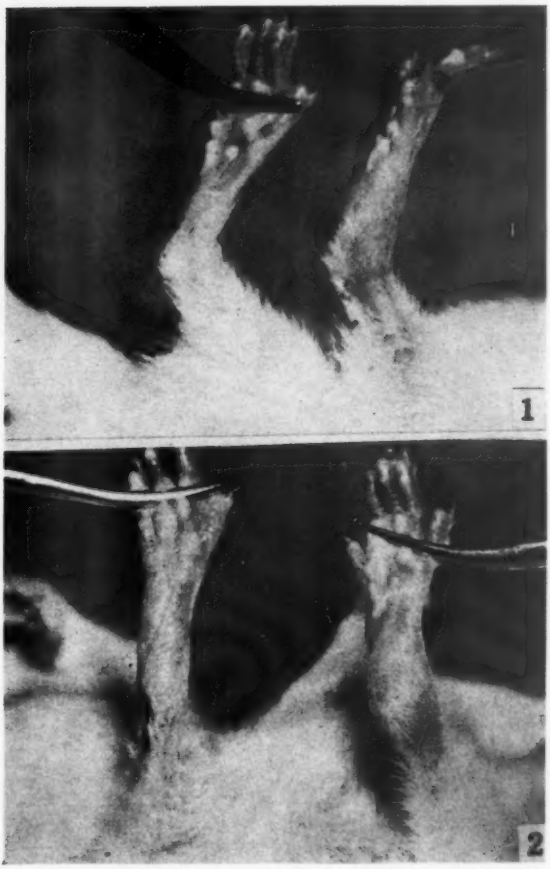

FIG. 1. Gross appearance, in lateral view, of the joint swelling (right) in one of a pair of rats in parabiosis for 21 days. Note the normal appearance of the foot (left) in the attached parabiont.

FIG. 2. Pronounced ankle swelling, seen in ventral view, in one of a parabiotic pair (right) contrasted with the normal joint (left) of its partner. Pair in parabiosis for 17 days.

FIG. 4. Marked arthritis with the projection of a greatly inflamed synovial membrane into the joint cavity. Note the swollen appearance of the membrane due to a proliferative granuloma. X 42. Haematoxylin and eosin.

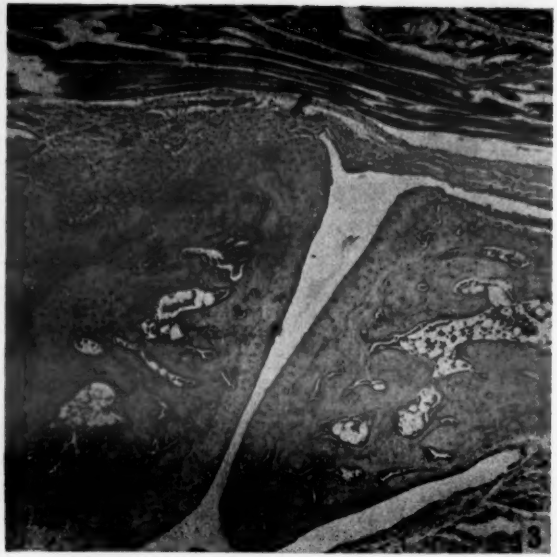

FIG. 3. Normal joint structure in a parabiotic rat. The synovial membrane, connective tissue, cartilage and joint cavity are devoid of any pathologic change. X 42. Haematoxylin and eosin.

FIG. 5. Fibrinoid necrosis and granuloma formation adjacent to a joint. Note the pannus formation in the joint cavity. X 42. Haematoxylin and eosin.

FIG. 6. A marked inflammatory response in the connective tissue between the periosteum and the muscle in the metatarsal region. In the center, the tendency of the muscle fibers to be disrupted by proliferating granuloma tissue and to undergo degenerative changes is apparent. X 42. Haematoxylin and eosin.

regions was also commonly observed. Muscles in the metatarsal regions were frequently surrounded and infiltrated by inflammatory cells. In such areas the continuity of the muscle fibers was frequently interrupted and the intervening regions were occupied by invasive inflammatory cells. In still more chronic cases, actual erosion of the joint cartilages was observed.

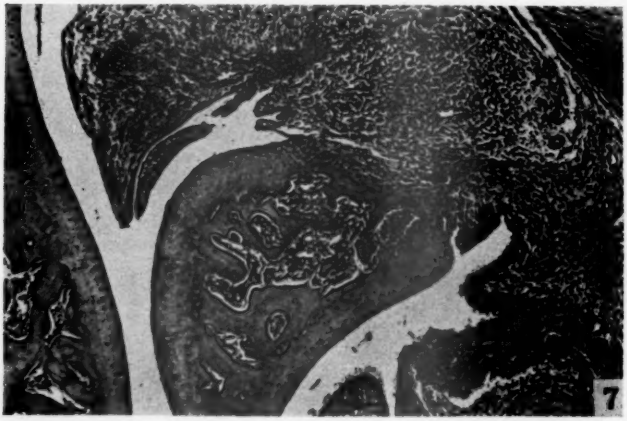

FIG. 7. Destruction of the surface cartilage, by actively proliferating granuloma tissue, in a metatarsal bone. X 42. Haematoxylin and eosin.

Discussion

It is evident that rats placed in parabiosis may develop all of the pathologic changes which result from DCA overdosage. In connection with the arthritic manifestations it is interesting to note that while many investigators have failed to observe arthritis in DCA-treated rats; and while others who have been more successful have commented upon the inconstancy of this particular manifestation, at least one laboratory has reported that histologic evidence of arthritis is invariably found in adrenalectomized, but not in intact, DCA-treated rats. This would suggest that a high mineralocorticoid/glucocorticoid ratio predisposes to arthritis: indeed this is probably also true of the cardiovascular lesions produced by DCA, since these may be inhibited by carbohydrate active adrenal steroids (Woodbury).

In the previous papers cited, it was suggested that the syndrome of parabiotic hypertension may be conditioned or augmented by an adrenal hyperactivity with respect to mineralocorticoids. The cardiovascular lesions encountered in parabiotic rats are confined to hypertensive animals: the fact that the arthritic changes show no such predilection, would seem to indicate that the evocative stimulus for the production of these lesions differs either qualitatively or quantitatively. However, arthritis evoked by DCA overdosage usually precedes temporally, often by weeks, the development of hypertension. This would suggest that the joint is a more sensitive end organ to some of the effects of DCA than is the cardiovascular system, despite the fact that while all rats treated with excessive dosages of DCA ultimately develop hypertensive vascular disease, arthritic manifestations are only sporadic. Thus it may be that in many of our parabionts there develops a temporary, mild adrenal hyperactivity of the mineralocorticoid type, which is either of insufficient magnitude or duration to produce the hypertensive syndrome which may require a more intense and prolonged hyperactivity. The incidence might also be higher than has been indicated, since the feet of the parabionts were not examined microscopically, and we merely tabulated the grossly apparent lesions and made histologic preparations of these.

Not only is the arthritis produced by DCA separable from the hypertensive-cardiovascular syndrome by virtue of the fact that it may appear in treated rats before they become hypertensive, but also there is no evidence to indicate that the arthritic manifestations are potentiated by either uninephrectomy or increased sodium chloride intake as is so clearly the case with the hypertensive syndrome. Therefore the present results which indicate neither an augmenting effect of sodium chloride nor a restriction of the condition to hypertensive rats are not at variance with our opinion that the disease may well represent a type of adrenal cortical dysfunction.

A consideration of the relationship which exists between the cardiovascular hyalinosis diseases, arthritis and the adrenal cortex might be pertinent at this time. It is well established that horse serum allergy in the rabbit or serum sickness in

man, may be accompanied by manifestations of periarteritis nodosa. In rabbits rendered anaphylactic to horse serum, arthritis is occasionally observed; although because the joint involvement was present in untreated controls and in only 6 of 53 horse serum-treated rabbits, the authors concluded that ". . . the incidence was not sufficient to indicate a causal connection between these changes and the administration of horse serum." However, the possible association of arthritis and periarteritis nodosa in the hypersensitivity disease, their frequent association in DCA-treated rats and in untreated rats placed in parabiosis, suggests a relationship between the three states. Since DCA overdosage is obviously a condition characterized by a preponderance of circulating mineralocorticoid over glucocorticoid hormones, owing to adrenal cortical atrophy produced by the steroid, the possibility that this is the common etiologic agency may be envisaged. This thesis is strengthened by the observation that cortisone antagonizes both the hypertensive effects of DCA and the vascular damaging effects of horse serum hypersensitivity. Indeed this fundamental antagonism between DCA and cortisone is also exemplified by the fact that the former augments and the latter inhibits the development of both formalin induced "topical irritation arthritis" and the articular lesions observed to develop in scorbutic guinea pigs (Schaffenberg).

These observations lend support to the contention that mineralocorticoids may either initiate the hyalinosis diseases or render the target organs more sensitive to other agents which do so; whereas glucocorticoids exert a protective effect. Our observations that a large proportion of parabiotic rats, otherwise untreated, develop either cardiovascular disease or arthritis, and occasionally both together, may be referable therefore to a reaction to the parabiotic state. In the affected animals this is characterized either by a preponderance of adrenal mineralocorticoid response over glucocorticoid production or by a systemic condition which sensitizes the target organs to mineralocorticoid action. Of the systemic conditions which might produce such changes, that of allergic hypersensitivity would appear to be the most likely.

Summary

When female rats are placed in parabiosis, arthritis is frequently observed to develop in the tarso-metatarsal region. Sodium chloride has no potentiating effect upon the incidence or severity of the lesion, nor is it confined to rats which develop hypertensive cardiovascular disease. The similarity of the lesion to that produced by desoxycorticosterone overdosage is discussed and the view is expressed that the lesion might reflect a mineralocorticoid hypersecretion.

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A METHOD FOR STUDYING THE EFFECT OF P^{32} ON LIVING ADULT HUMAN EPIDERMAL CELLS IN A PERFUSION CHAMBER*

F. N. Hu, S. G. Holmes, C. M. Pomerat,
C. S. Livingood, and K. P. McConnell

Of the various methods which have been suggested for the analysis of biological radiation effects upon cell constituents, the technique of tissue culture is significant in offering preparations which can be observed in the living state under highly controllable conditions in the course of applying experimental variables. This field has been recently reviewed in two excellent reports by Buchsbaum and by Brues & Stroud in a chapter dealing with tissue culture methods which forms a part of Volume IV of "Methods in Medical Research" (Academic Press, 1951).

The purpose of the present study is to report provisional experiments on the effect of P^{32} on living adult human epidermal cells when introduced in a nutrient fluid within a perfusion chamber designed to permit still and motion picture photographic recordings of cellular structure. The study is preliminary in nature but designed to illustrate the feasibility of the method with respect to observations upon adult human tissues.

Materials and Methods

Adult human skin explants were cultivated *in vitro* with the technique described in previous publications (*cf.* Everett, Pomerat, Hu, and Livingood, 1951). The cultures were made as hanging drop preparations and incubated at 37° C. for 4 to 7 days. When maximal outgrowth was obtained, cultures were transferred to perfusion chambers (fig. 1) designed to permit continuous observation of the effect of various test solutions upon the activities of the cells.

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*From the Departments of Dermatology and Biochemistry, and the Tissue Culture Laboratory, Medical Branch, The University of Texas, Galveston, Texas. Aided by grants from Smith, Kline & French and from Hoffmann-La Roche, Inc. Received for publication August 15, 1951.

The basic fluid utilized as a control perfusate consisted of equal parts of Gey's balanced salt solution (Gey and Gey, 1936) and ascitic fluid. Radioactive phosphorus was incorporated into Gey's solution in the form of sodium phosphate for experimental analyses. Sodium-phosphate-free Gey's solution was made at a concentration 5.5 times higher than the regular Gey's balanced salt solution and rediluted with distilled water to its normal concentration after the radioactive sodium phosphate was added.

In these experiments, P^{32} was used in the form of phosphoric acid; 1 ml of this material being equivalent to 1.3 mc. Altogether, 2 ml of the solution was used. This was neutralized with 0.42 ml of 1.04 N sodium hydroxide to form sodium phosphate which raised the total volume to 2.42 ml. To this, 1 ml of concentrated Gey's solution and 2.08 ml of distilled water were added to make a total of 5.5 ml. Thus the final solution was a radioactive Gey's solution with all of its constituents in normal proportions. The activity of the perfusion fluid was calculated as follows:

> Total volume of the stock solution is 5.5 ml. The concentration of P^{32} in the solution is 2.6 mc/5.5 ml or 0.47 mc/ml. As the perfusion fluid consisted of only half of the amount of the stock solution, the final concentration of P^{32} in the perfusion fluid was 0.47 mc/2 ml or 0.24 mc/ml.

Since the purpose of the present report is merely to demonstrate a method of investigation, no attempt is here made to work out calculations of the actual amount of radiation received by the cells. Suitable quantitative methods will be used in assembling data essential for precise calculations. Further dilutions were made of the stock radioactive Gey's solution with the addition of regular Gey's solution.

After skin cultures were transferred to the perfusion chambers, they were irrigated overnight with the control solution consisting of equal parts of ascitic fluid and regular nonradioactive perfusion fluid. On the following morning, several control pictures were taken of the epidermal cells at 15 minute intervals with phase contrast photomicrography. The test

perfusion fluid was then introduced and pictures were taken at intervals of 15 minutes, 1 hour or 2 hours, depending upon the rate of change in cellular activity. Thus the progressive injury from beta radiations upon the normal human epidermal cells could be recorded.

Results

Table I summarizes the essential findings obtained in preliminary experiments. Under the conditions reported, beta radiations produced rounding of epidermal cells within 15 to 90 minutes. No evidence of recovery was found even 22 hours after washing treated cells with control perfusion fluid. The time necessary to elicit such injury effects seemed to be grossly proportional to the doses used.

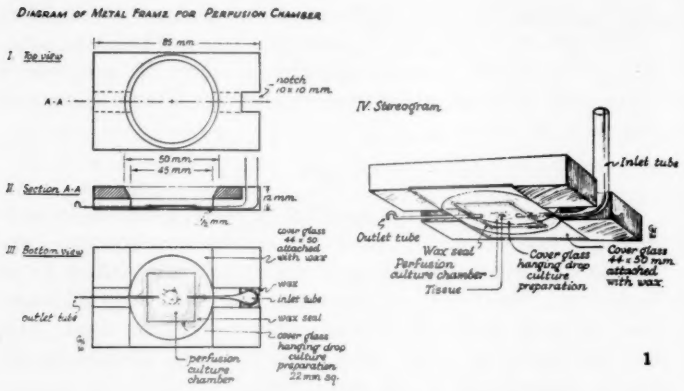
TABLE I

Preliminary experiments on the effect of radioactive phosphorus (P^{32}) upon tissue cultures of adult human epidermal cells in a perfusion chamber.

Exp. No.	Age of Cultures	Perfusion fluid* mc/ml	Results
I.	9 days	(1:1) 0.24	Retraction and rounding of cells in 15 min. No recovery after 22 hours.
III.	4 days	(1:10) 0.024	Retraction and rounding of cells in 15–30 min. No recovery after 20 hours.
IV.	5 days	(1:100) 0.0024	No change after 20 hours.
VII.	5 days	(1:20) 0.012	Retraction and rounding of cells in 30–90 min.
VIII.	4 days	(1:40) 0.006	No change after 4 hours. Slight retraction after 26 hours. No rounding of cells.
IX.	4 days	(1:30) 0.008	Retraction and rounding of cells in 17 min.

*Dilutions of the stock solution are shown in parentheses in association with values for millicurie per ml.

Representative illustrations of effects produced upon epithelial sheets are shown in figures 2–7. In figure 2, an emigrating group of elements was photographed immediately before the introduction of the isotope-containing solution used in Experiment IV, while the same cells 90 minutes after the



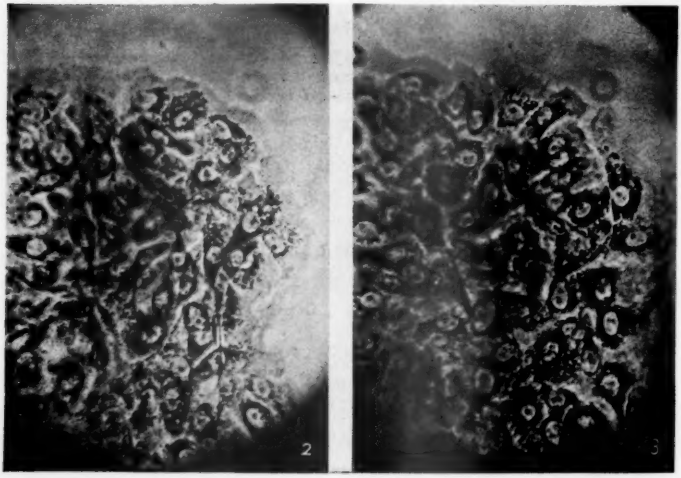


PLATE I

FIG. 1. Diagrams of a metal frame used for a perfusion chamber showing the arrangement of the assembly.

FIGS. 2 and 3. Tissue culture of adult human epidermis used to study the effect of P^{32} in a perfusion solution. Both illustrations were made with an 8 mm dark phase contrast objective at approximately 300x.

FIG. 2. Appearance of the culture following 14 hours of perfusion with nutrient solution not containing the isotope.

FIG. 3. The same culture as shown in figure 2 but 90 minutes after the addition of perfusate containing P^{32} at 0.0024 millicurie per ml.

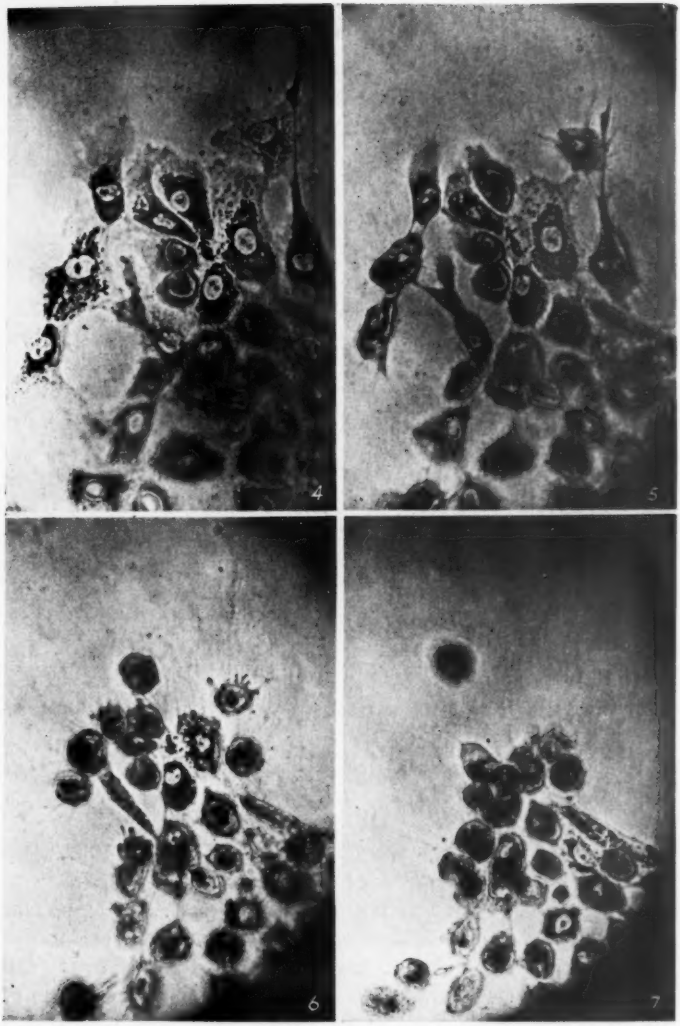


PLATE II

FIGS. 4 to 7. Tissue cultures of adult human epidermis showing injury effects resulting from contact with P^{32}. All figures were made with the use of an 8 mm dark phase contrast objective at approximately 300x.

FIG. 4. Appearance of culture following 14 hours contact with control perfusate.

FIGS. 5 to 7. Progressive injury following perfusion of culture with fluid containing P^{32} at 0.012 millicurie per ml, after 15 minutes (fig. 5), 30 minutes (fig. 6) and 60 minutes (fig. 7).

experimental perfusion are shown in figure 3. In figure 4, the culture used for Experiment VII is represented as it appeared before coming in contact with radioactive P^{32}, while figures 5, 6, and 7 represent injury effects following contact for 15, 30, and 60 minutes, respectively, after the introduction of the isotope.

Discussion and Summary

The results which have been obtained thus far have established that with the use of a technique successful for the cultivation of epidermal cells from adult human skin, it is possible to record the effect of various concentrations of radioactive phosphorus as a function of time, upon the appearance of living cells maintained in a perfusion chamber. With this technique, the concentration of P^{32} required to produce irreversible injury within an 8-hour period may be easily determined. Knowing this value, it will be possible to observe the character of the injury changes in greater detail. The proposed plan for recording radiation effects upon living cells in perfusion chambers not only involves static photomicrography but also a setup which has been developed for cinematographic recordings, using 35 mm film in connection with a 1.9 mm phase contrast objective system. Figure 8 illustrates the microscope-camera setup.

In previous papers from our laboratories, general statements have been made regarding the appearance and activities of living human epidermal cells. Of special interest with respect to cellular dynamics is the movement of the free margin of the cells. Figure 9 shows the character of cytoplasmic irregularities associated with what is believed to represent the process by means of which nutrient materials are swept into the interior of the cytoplasm. Under the term pinocytosis (cell drinking), W. H. Lewis (1931) explained the appearance of vacuolar systems such as those shown in figure 9 as a part of the process by means of which cells can assimilate relatively large colloidal aggregates. This process is regularly observed in tissue cultures of human skin (Lewis, Pomerat, and Ezell, 1949). Recalling estimates made by Lewis (1931) that cells may take up approximately one-third of their volume of the

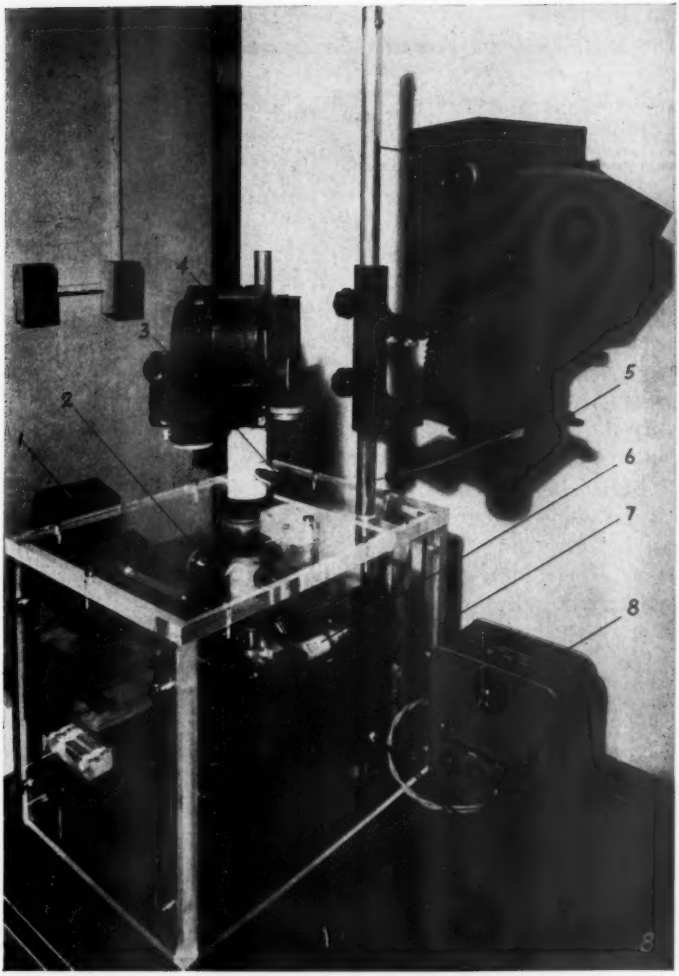


PLATE III

FIG. 8. Setup for still and motion picture photography of human cells in a tissue culture perfusion chamber used for studying the effect of isotopes.

1. Light source.
2. Microscope carrying phase contrast optical system.
3. Periscope.
4. 35 mm motion picture camera.
5. Camera for "still" photomicrography.
6. Heating element.
7. Lucite incubator wall.
8. Thermo-regulator unit.

Both the movie and still cameras can be swung over and away from the ocular of the microscope.

surrounding fluid in 1 hour by the process of pinocytosis, the importance of this mechanism in relation to the uptake of the radioactive substance is of great moment.

Of special importance in the study of the morphological counterpart of intracellular metabolic activity are the mitochondria whose association with enzymes are now fully established. Epithelial derivatives from the human skin reveal the presence of fllamentous mitochondria whose long axes are generally arranged at right angles to the free margin of the cell (figure 10). Time-lapse cinematographic studies of such long mitochondrial filaments show them to be highly mobile, migrations to and from the periphery of the cell together with rotatory and bending movements being regularly observed.

Macrophages which are generally found in tissue culture preparations are recognized to be of considerable importance to the reparative activities of the skin. Figure 11 illustrates some of the form variations seen in macrophages in an area adjacent to an emigrating sheet of epithelial cells. A single, living macrophage exhibiting characteristic multipolar membrane activity is shown in figure 12.

Future studies directed at elucidating the effect of radioactive substances upon epidermal cells should include detailed cinematographic records of changes in membrane activity and

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PLATE IV

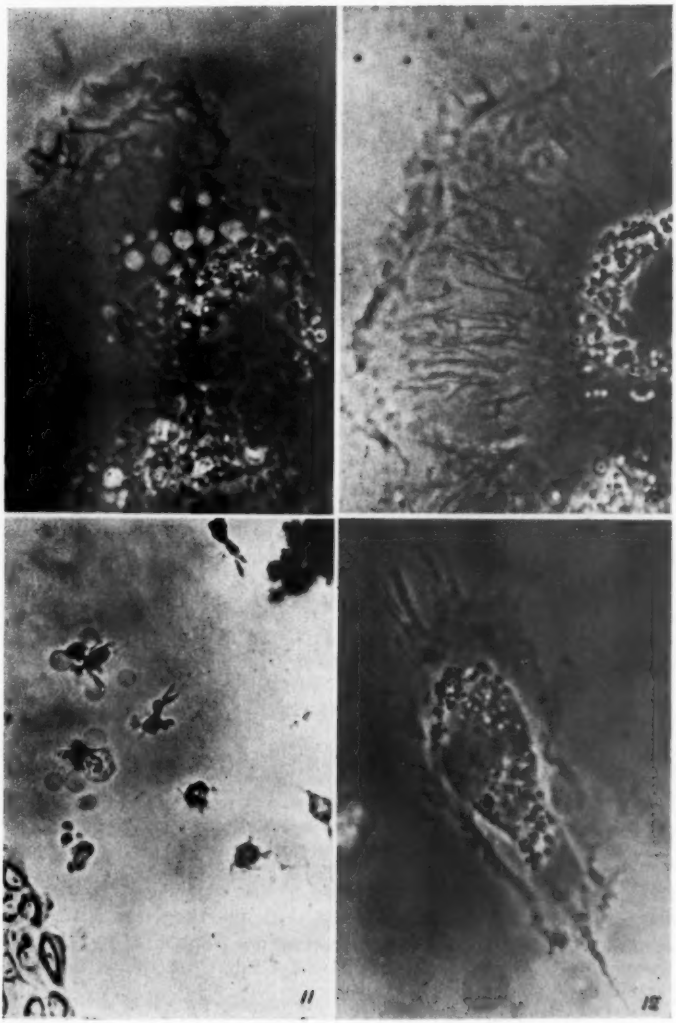
FIGS. 9 to 12. Cells from adult human skin in tissue culture. Figures 9, 10 and 12 were taken with a 1.9 mm dark phase contrast objective and represented at approximately 1500x. Figure 11 was made with an 8 mm dark phase contrast objective and reproduced at a magnification of 250x.

FIG. 9. Margin of an epidermal cell showing pattern representing membrane activity and globules resulting from the entrapping of surrounding fluid (pinocytosis).

FIG. 10. Epidermal cell showing filamentous mitochondria whose long axes tend to be arranged at right angles to the margin of the cell. Note the granular accumulations in juxtar nuclear zone at the right-hand edge of the photograph.

FIG. 11. A group of macrophages showing form variations in an area close to an epithelial sheet which can be seen in the lower left margin.

FIG. 12. A single macrophage at high magnification of the type found in areas as represented in figure 11. Note the pattern of the multipolar undulating membrane and the presence of perinuclear granules.



pinocytosis, disturbances in the pattern of mitochondrial movement associated with their ultimate destruction, and a description of the influence of radiations upon the scavenger function of the macrophages.

The challenge offered by the tissue culture method in exploring radiation effects upon the parts of the body which might be readily reached by radioactive dust is further enriched by the success which has been obtained in the cultivation of the human nasal mucosa (Rose, Pomerat, and Danes, 1949), pulmonary epithelium (Danes, 1949) and the cornea of laboratory animals (unpublished data).

The question of whether irreversible injury to the exposed epithelial cells depends upon a progressive succession of damage to cell organs or whether some single vulnerable locus of injury is involved may be answered with the techniques suggested in the body of this report.

Acknowledgment

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THE INACTIVATION OF POLIOMYELITIS VIRUS BY FREEZE-DRYING*

MORRIS POLLARD

The extraction of water from frozen suspensions of virus infected tissues *in vacuo* is a routine procedure for the preservation of viral agents. Such treatment obviates the necessity for a constant supply of dry ice in which some hydrated viral specimens must be stored. If the potency of a virus must be maintained for routine neutralization tests, then storage at dry ice temperature is indicated; however, for the mere survival of stock virus strains, many of them can be stored at 4° C. in 50% buffered glycerin or as tissue suspensions desiccated while in the frozen state.

Poliomyelitis virus is a relatively stable agent. In infected tissue it can be stored for years in 50% glycerol at 4° C. (Rhoads, 1929). Within a pH range of 3–9 and at 11° C. the virus will survive for at least 11 weeks; at 35° C. it will survive for at least one week (Pollard and Connally, 1949). It will tolerate treatment with di-ethyl ether (Clifton *et al.*, 1931) and will survive for weeks in feces and in sewage from cases or carriers of the virus (Kling *et al.*, 1912; Paul *et al.*, 1939). It is surprising, therefore, that poliomyelitis virus should be destroyed when freeze-dried, a process which preserves the virulence of more labile neurotropic agents such as rabies, Western and Eastern equine encephalomyelitis, St. Louis encephalitis viruses and others.

Specific viral complement-fixing antigens have been prepared from infected nerve tissues by their desiccation and extraction with benzene (DeBoer and Cox, 1947; Casals, 1949). When suspensions of MEF_1-infected mouse nerve tissues were subjected to such treatment no complement-fixing antigen could be consistently detected with serums which fixed complement with antigens prepared by other methods (Pollard, 1951, a & b). In view of this effect of drying on the virus, a systematic study

*From the Virus Research Laboratory, The University of Texas Medical Branch. Aided by a grant from the National Foundation for Infantile Paralysis, Inc. Received for publication August 16, 1951.

was made of what might be done to minimize such destruction of the virus.

Methods

Pooled nerve tissues of mice and cotton rats, infected with Lansing-type virus, monkey tissues infected with Brunhilde type (Kosh) virus and mouse tissues infected with TO encephalomyelitis virus were emulsified in physiological saline to make a 20% emulsion by weight. A commercial model freeze-drying apparatus† was employed to study the effect of such desiccation on: Saline emulsions of virus—crude, and clarified by centrifugation at 5,000 rpm for 20 minutes. Saline emulsions of virus mixed with "protective" agents such as serum, mucin, milk, amino acids, carbohydrates and bacteriological media (thioglycollate medium).‡ In certain instances the pH of the suspensions were intentionally altered.

Mouse nerve tissue emulsions of rabies (CVS strain) and St. Louis encephalitis viruses were similarly processed as a control measure on the free-drying technique. The ampouled test material was shell-frozen in a dry ice-alcohol mixture and immediately attached to the evacuated drying apparatus. When the frost disappeared from the exterior of the ampoule, coincident with drying, the process was continued for 2 additional hours. The dried preparations were immediately rehydrated with distilled water and decimal dilutions thereof (along with the unprocessed controls) were tested for potency by inoculation intracerebrally into mice (0.03 cc) or cotton rats (0.05 cc). All animals were examined daily during the 21 days following inoculation for evidence of infection. Each test was repeated at least once.

The effect of desiccation on the immunogenic properties of poliomyelitis virus was determined in monkeys by inoculating unprocessed and dried-rehydrated MEF_1 Lansing-type virus (mixed in an oily emulsion§) intramuscularly each into 2 monkeys (*Macacus mulatta*). After two intramuscular inoculations of 1,000 monkey LD_{50} of virus at intervals of 2 weeks

†Cryochem apparatus manufactured by Stokes Machine Company, Philadelphia, Pa.

‡Baltimore Biological Laboratories, Baltimore, Md.

§Pendil—manufactured by Endo Products Company, Richmond Hill, N.Y.

they were challenged 4 weeks after the last inoculation with 100 monkey LD_{50} of the same virus intracerebrally. The monkeys were observed thereafter for 21 days for evidence of infection.

Results

When poliomyelitis virus is freeze-dried, its infectivity is reduced to the extent of 2–3 logs. None of the added protective agents could prevent this inactivation of virus (Table I). Gas-

Table I

EFFECT OF FREEZE-DRYING OF POLIOMYELITIS VIRUS ON SURVIVAL OF VIRULENCE

Virus	CNS Tissue	Adjuvants	pH	Control at 5°C. 10^{-1}	10^{-2}	10^{-3}	10^{-4}	Desiccated 10^{-1}	10^{-2}	10^{-3}
MEF_1	Mouse	0.15% NaCl		7/7*	7/7	4/6	1/4	6/13	2/10	0/7
		50% Rabbit Serum		6/6	7/8	7/8	0	0/7	0/8	0
		6% Glucose						0/1	0/4	0/4
		12.5% Lactose	6	3/3	3/3	0	0	2/3	0/3	0
		1.5% Casein Amino Acid	7.0	5/6	6/6	0	0	0/7	0/9	0
		3% Skim Milk		7/7	7/7	4/6	1/4	1/3	0/4	0/4
		0.5% Tryptophane	4.5	3/3	3/3	0	0	0/3	0/3	0
		0.5% Tryptophane	7.0	3/3	3/3	0	0	0/3	0/3	0
		5.0% Gastric Mucin		6/6	8/8	5/7	0	7/10	4/11	0
		Thioglycollate Broth		3/3	4/4	3/3	1/3	6/8	0/8	0
TO	Mouse	0.15% NaCl		7/7	8/8	6/7	0	2/7	0/8	0/4
Lansing	Cotton Rat	0.15% NaCl		4/5	5/5	4/5	3/5	10/10	4/10	0
Kosh	Monkey	0.15% NaCl		3/3	3/3	3/3	3/3	0	0/2	0

* Number paralyzed/number inoculated

0 Not tested

tric mucin appears to have protected the virus; however, subsequent tests indicate that such was the exception rather than the rule. The effect of drying is the same with Lansing, MEF_1 and Kosh strains of poliomyelitis viruses, as well as with the TO strain of mouse encephalomyelitis virus. pH adjustments of the virus suspensions prior to drying did not alter the above results. While approximately three logs of virus were lost from Lansing-infected cotton rat tissues by drying, the agent

could frequently be recovered from 10^{-1} dilutions of such dried material.

The two monkeys inoculated with MEF_1 virus in "Pendil" were immune to cerebral challenge with 100 monkey LD_{50} of the same virus. Dried and rehydrated virus mixed with Pendil was not immunogenic (Table II) and the two monkeys inoculated with such material developed the disease following the challenge inoculations. Rabies and St. Louis encephalitis viruses were not inactivated by freeze-drying and their antigenic properties appeared to be unaltered.

TABLE II

Influence of desiccation on immunogenic property of MEF_1 virus in monkeys.

Two intramuscular inoculations at 4 week intervals. All monkeys challenged 4 weeks later with $100LD_{50}$ MEF, virus intracerebrally.

Inoculum	Immunization dose total LD_{50}	Challenge result $100LD_{50}$ intracerebral
MEF. & pendil	2000	Immune
MEF. & pendil	2000	Immune
MEF. & saline	2000	Paralysis
MEF. & saline	2000	Tremors, excitable*
MEF. dried & pendil	0	Paralysis
MEF. dried & pendil	0	Tremors, excitable*

*Cord showed microscopic lesions in poliomyelitis.

Discussion

Few other viruses have been reported as being labile to desiccation and most of these have been of plant origin: bushy stunt, tobacco ringspot, and potato "y" viruses (Bawden, 1943). The virus causing Sao Paula parrot disease appears to deteriorate by drying (Pacheco, 1932). Poliomyelitis virus is inactivated as soon as it is dry and this response is not prevented through the addition of so-called "protective" agents such as milk, mucin, carbohydrates and others (Table I). The freeze-drying technique employed in this study can be considered suitable since it caused no detectable loss of virulence with rabies and St. Louis encephalitis viruses.

Concomitant with the marked attenuation of poliomyelitis virus when dried, there seems to be a change in its immuno-

genic property. The immunity enhancing effect of a depot-producing agent ("Pendil") was inadequate to compensate for the amount of surviving virus in the inoculum. By conventional methods it is difficult to immunize monkeys against poliomyelitis. By mixing virulent virus with "pendil" it is possible to engender a cerebral immunity from 2 intramuscular inoculations. When "pendil" was omitted, numerous intramuscular inoculations were required for the same response, as indicated by Morgan *et al.*, 1947. It is apparent in Table II that MEF_1 poliomyelitis virus is not as highly immunogenic as it is when mixed with "pendil."

Dried Lansing virus of cotton rat origin occasionally contained small amounts of viable virus. This might be attributed to its high initial level of virus which was not sterilized by a 3–4 log drop in virulence, or perhaps to incomplete dehydration of some specimens. In 1909, Landsteiner and Levaditi recorded the preservation of poliomyelitis virus by drying cord emulsions of it over KOH. These results were confirmed by Romer and Joseph (1910) but not confirmed by Liener and von Wiesner (1910); however, the techniques of the latter appeared to be too drastic for the results to be significant. It is probable that Landsteiner and Levaditi (1909) did not dry the virus as thoroughly as is being done by the Freeze-drying apparatus.

The catalog of the American Type Culture Collection, Viral and Rickettsial Registry, 1950, recommends that poliomyelitis virus not be stored in the dried state. Many investigators of this virus may have realized this matter for years even though extensive studies of it have not appeared in the literature. Recently, Faber and associates (1951) in describing the inactivating effect of drying the virus in nasopharyngeal secretions and in stools, postulate the possible epidemiological significance of such findings.

Summary

In view of the data herein recorded and of the reported observations of others, it would not be advisable either to attempt the preservation of virulent poliomyelitis virus by conventional freeze-drying, or to contemplate the production

of dried vaccines thereof. Freeze-drying preserves neither the virulence nor the antigenicity of poliomyelitis virus.

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INTRARENAL PRESSURES DURING SUBACUTE CONGESTIVE HEART FAILURE*

J. M. Prine, Lee Roy Mathis, and H. G. Swann

Recent studies have brought forward considerable evidence concerning the relation of the kidney to congestive heart failure. The presence of a decrease in glomerular filtration rate, in renal blood flow, in sodium and water excretion, and other disturbances in renal physiology are generally agreed upon, but the causative mechanisms and sequence in which these factors appear are subject to much speculation and debate. Some evidence favors the hypothesis that the lowered filtration rate causes Na retention (Stead, 1949; Merrill, 1949); other evidence suggests that the lessened renal blood flow, caused by an elevated venous pressure, is primarily responsible (Bradley and Blake, 1949). Blake, Wegria, Keating, and Ward (1949) demonstrated that an increase in renal venous pressure to 340 mm. saline causes sodium retention in dogs without however changing the filtration rate or the renal plasma flow. Selkurt, Hall, and Spencer (1949) and Hall and Selkurt (1951), imposing venous pressures of 30 cm. of water on the kidney, also reported that the renal blood flow and filtration rates were not conspicuously changed.

However, Hall and Selkurt (1951) usually found, as did Blake *et al.* (1949), a sodium and water retention under this venous hypertension. They were investigating the behavior of acute preparations. When essentially the same experiment was done in chronic preparations, Hwang, *et al.* (1950) found a similar change postoperatively, but within a week, in spite of continuing high venous pressures, sodium and water excretion returned to normal. The effect of venous pressures, then, is certainly real, but it appears to be transitory.

In explanation of these results, Bradley and Blake (1949) suggested that the renal engorgement caused by the increased

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venous pressure somehow influences water and sodium reabsorption. The engorgement, they suppose, should be accompanied by "an increment in renal interstitial pressure approximately equal to that in venous pressure." But this hypothesis appeared to us unlikely because the IRP of dogs has recently been shown to be about 26 mm. Hg (Montgomery, Davis, Prine, and Swann, 1950), or far higher than the venous pressures characteristic of congestive heart failure. In experimental congestive failure, as produced by Fishman, Stamler, Katz, Miller, Silber, and Rubenstein (1950), by means of chronic pericarditis with effusion, the venous pressure rises only to about 9 mm. Hg. This pressure would not, presumably, be transmitted back into a region where the pressure is 26 mm Hg. Nevertheless, it seemed possible that the intrarenal pressure might change during congestive failure, and in especial it seemed possible that it might actually fall. It might fall to, say, 8 mm. Hg (11 cm. H_2O), thus allowing, as it were, the venous pressure to influence renal function and to produce the very conditions which Bradley and Blake postulate as facilitating sodium and water retention.

New methods for producing congestive failure and for measuring intrarenal pressure afford a means for testing this hypothesis and the theory of Bradley and Blake. Experimental congestive failure was therefore produced in dogs by pericarditis and pericardial effusion, and the intrarenal pressure was then measured as fatal congestive failure developed.

Methods

Adult male and female dogs were used. The right kidney was removed and the left kidney explanted to a subcutaneous position in the flank. After recovery from this procedure a series of measurements of blood pressure, venous pressure, and intrarenal pressure were made, all of these being taken on trained unanesthetized dogs. Blood pressure was measured by femoral artery puncture and recorded optically using a Bourdon tube manometer (Kubicek, Sedgwick, and Visscher, 1941; Swann and Brucer, 1949). The venous pressure was taken in the left cephalic vein with a saline manometer. This measurement was taken with the dog lying on its right side, using a

point 6 cm. below the top of the thorax as a reference point. The intrarenal pressure (to be designated as IRP) was measured by means of the transduced equilibrium method of Swann, Montgomery, Davis, and Mickle (1950). A 20-gauged syringe needle, with the end plugged and five small holes bored in the shaft, was inserted through the skin and deep into the renal parenchyma. Then a small drop of saline was forced through the needle and into the kidney by a Bourdon tube manometer under an initial pressure of 150 mm. Hg. When no more fluid was forced in by the manometer, equilibrium with the tissue pressures was reached. This is the intrarenal pressure. The fluctuations in the manometer were recorded optically, thus giving an objective measure of the IRP.

After a series of normal values were obtained on each dog, the method of Fishman *et al.* (1950) was used to produce pericarditis and pericardial effusion. The chest was entered through the fifth left interspace, and the pericardium was incised longitudinally about 2 cm. medial to the left phrenic nerve. By use of two large aneurism needles a bag of "polythene" cellophane† was slipped around the heart beneath the parietal pericardium. The pericardium was closed with continuous sutures and several interrupted sutures were inserted to fix the bag in position. Measurements of blood pressure, venous pressure and IRP were made approximately every second day until death.

Results

Determinations were made on four dogs. The data are shown in Figure 1 in which are given the serial measurements of systolic and diastolic blood pressures, the IRP and the venous pressures for each of the dogs up until death. All dogs recovered from the chest operation in a few days and appeared in good health until a few days before death. The course of events in these dogs followed exactly the pattern described by Fishman *et al.* for similar preparations. Following surgery all dogs but one showed a decreased pulse pressure and all showed

†Supplied through the courtesy of the Film Department of the E. I. du Pont de Nemours and Co., Wilmington, Del.

a tachycardia which became more severe before death. Peripheral venous engorgement was apparent after the fifteenth day in all animals except No. 3, and respiratory distress appeared at this time, the breathing becoming extremely labored as death approached. Venous pressures slowly rose up to about 15 mm. Hg (20 cm. saline). Ascites was present after the

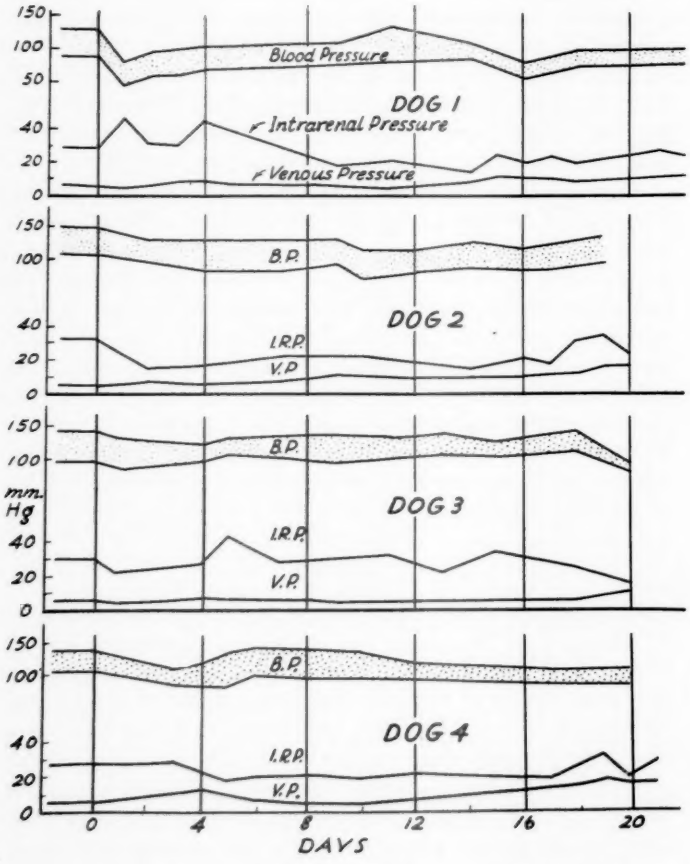

FIG. 1. Arterial, venous and intrarenal pressures during subacute congestive failure.

sixteenth day in all animals, but peripheral edema was not evident at any time. Death occurred at 21 days in two dogs and at 22 days in the other two. Anorexia was present for three to four days preceding death.

At autopsy there were present 400–1,200 ml. of clear ascitic fluid. The viscera showed signs of venous engorgement. The kidneys appeared only moderately congested and were not enlarged, but the renal vein was distended in every case. The thorax contained 100–600 ml. of blood-tinged transudate and the lungs were congested, two or more lobes being completely engorged in every animal. The pericardial cavity contained from 100–800 ml. of thick bloody fluid in three of the dogs. Dog No. 1 had a large organized clot completely filling the pericardial cavity. In dog No. 3 the bag had partially slipped off the heart; the distended pericardium filled approximately one-half the thoracic cavity and contained 800 ml. of moderately clear fluid. The hearts were all small and appeared normal except for several fibrous tags on the ventricles.

With respect to the IRP, clear-cut elevations did not occur during the course of the fatal congestion. The trend, dog No. 3 excepted, was downward: the IRP fell to roughly half the control level for the first two postoperative weeks. Then there occurred a terminal rise. In No. 3, in which the cellophane bag slipped off the heart, the rise in venous pressure and fall in IRP occurred only very late. However, the important fact established in the present experiment is that the venous pressure did not exceed the IRP in any of the dogs at any point during the development of the fatal congestive episode. Even though the venous pressure showed the increase characteristic of congestive heart failure, the IRP was found always to be well above the venous pressure.

Discussion

That the pericarditis causes a retention of fluid was shown by the pronounced ascites, hydrothorax, and venous engorgement observed in the dogs at post-mortem examination. Furthermore, this is known to be accompanied, as clearly shown by Fishman *et al.* (1950), by a retention of sodium.

The finding that the IRP does not rise in congestive failure fails to confirm the hypothesis of Bradley and Blake (1949) that the rise in venous pressure during congestive failure may elevate the IRP. This finding, as mentioned above, was not unexpected because we have recently found that the IRP of dogs is about 26 mm. Hg (Montgomery *et al.*, 1950). The presence of such a pressure in the renal parenchyma presumably necessitates at least as high a pressure in the various elastic fluid-filled compartments of the kidney (*e.g.*, capillaries, venules, and tubules) if flow through them is to take place. Unless, then, the IRP is exceeded by the venous pressure during congestive failure, it was thought unlikely that IRP would be raised. Furthermore, it has also been found (Swann, Montgomery, and Lowry, 1951) that acute elevation of the renal venous pressure does not influence IRP until the venous pressure exceeds IRP. At this point the two pressures rise together in a one-to-one relationship. These recently discovered facts, therefore, made it unlikely that the elevation of venous pressure which is characteristic of congestive failure would change the IRP. The experimental results confirmed this line of reasoning: even though the venous pressure rose, it did not rise above IRP and so no change in IRP took place.

Similarly, an elevated renal venous pressure, provided it is less than IRP, does not influence renal plasma flow or the glomerular filtration rate, as demonstrated by both Blake *et al.* (1949), and Selkurt *et al.* (1949). It required, in the experiments of Blake *et al.*, a pressure of 40 mm. Hg (550 mm. saline)—some 14 mm. more than IRP—to decrease flow and filtration. The explanation of Bradley and Blake (1949) appears to us to be quite correct, provided it is applied to pressures greater than IRP. At venous pressures of 40 mm. Hg, the IRP having been overcome, there occurs a backing-up of blood into the peritubular vessels and therefore peritubular congestion, with decreased blood flow. The congestion might be transmitted back beyond the efferent arteriole and thus lower the filtration rate; or it might compress the renal tubules, thus increasing the resistance to tubular flow which in turn would increase intracapsular pressure. This also would reduce the filtration rate.

Even the data of Fishman *et al.* (1950) confirm this hypothesis. Although they state that the elevations in venous pressure of experimental constrictive pericarditis are accompanied with reductions in filtration rate and blood flow, these, it is said, occurring late during failure, their data are not convincing. Of the 5 dogs in which the filtration rate was observed, 2 had increased rates, 2 had mild, but insignificant decreases, and 1 showed a pronounced decrease. But the latter animal was moribund, with a blood pressure so low as to be unrecordable.

Similarly, the data on plasma flows are unconvincing: 3 dogs had elevations or insignificant changes, 1 was moribund, and only 1 of the group had a clear-cut fall. If anything, these data show that neither the flow nor the filtration rate change until the dog is moribund. The explanation is that the venous pressure never exceeds the IRP in this type of congestive failure. But it seems to us probable, in agreement with Bradley and Blake (1949), that in other types of congestive failure, especially the long-standing failures, the venous pressure exceeds IRP. In order for a venous pressure of 20–30 cm. H_2O (15–20 mm. Hg) to overcome the IRP, it is not unlikely that the IRP falls during the course of the disease. If in the present experiment, the congestive episode had been chronic instead of subacute, the IRP would presumably have continued to fall and eventually it would have fallen below the rising venous pressure. At this time the glomerular filtration rate and renal blood flow would start to decrease.

The present experiment, of course, does not show that sodium and water excretion are not influenced by the venous pressure. What has been shown is that the venous pressure does not exceed IRP; in fact it does not even approach the value of 18 mm. Hg which was shown by Blake *et al.* (1949) to induce sodium retention. Thus it has been impossible to ascribe the recognized changes in sodium and water excretion to elevations in renal venous pressure or in IRP. Indeed, speaking teleologically, one of the functions of IRP, uniquely high for a tissue pressure as it appears to be (Montgomery *et al.*, 1950), is to "protect" the kidney's peritubular vessels from the vena caval pressure. It is the characteristic compactness of the

kidney that gives the organ this high interstitial pressure. The IRP is "set high" in order that random fluctuations in the cava pressure, as in orthostasis or exercise, may not influence the absorptive and secretory process going on in and around the tubules. The IRP, in fact, appears to operate as a sort of prolonged efferent vessel; it takes, perhaps, the pressure of the efferent arteriole and evens it off all the way through the peritubular capillaries and venules. It gives a constant, not a fluctuating, pressure for the whole delicate interplay of tubular processes.

Summary

Measurements were made of the arterial, venous, and intrarenal pressure during the congestive failure caused in dogs by pericarditis with effusion. No elevation in intrarenal pressure was observed. The venous pressure never rose at any time during the fatal congestive episode to a level equal to or higher than the simultaneous intrarenal pressure. The relation between systemic venous pressure and intrarenal pressure is discussed.

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BLOOD LEVELS IN RELATION TO DOSAGE OF 3,4 DIMETHYL-5-SULFANILAMIDO-ISOXAZOLE (GANTRISIN) IN CHILDREN WITH CLINICAL OBSERVATIONS*

P. Clift Price and Arild E. Hansen

Although the new sulfonamide, 3,4-dimethyl-5-sulfanilamido-isoxazole (Gantrisin), has been used rather extensively for adult subjects, relatively little data are available concerning dosage and routes of administration for infants and children.

A significant feature of this drug is its high solubility in water and consequent low incidence of renal complications. For example, in a series of 300 patients, Svec, Rhoads, and Rohr (1950) observed no evidence of kidney damage. They demonstrated that in urine the solubility of Gantrisin rapidly increases from 60 mg. per 100 ml. at pH 5.4 to 327 mg. per 100 ml. at pH 6.1. The solubility of sulfadiazine at pH 6.3 is only 12.9 mg. per 100 ml. and approaches that of Gantrisin when the pH of the urine is raised to 7.5 or 8. About 70 per cent of the Gantrisin excreted in the urine is in the free form and this is the most soluble fraction.

Rhoads, Svec, and Rohr (1947, 1949, 1950) found Gantrisin to compare favorably with other sulfonamides in a variety of bacterial diseases. For example, these investigators (1950) reported that all ten patients with meningococcus meningitis treated with this drug alone recovered. The spectrum of effectiveness is essentially the same as for other sulfonamides. Except for a report by Rodgers and Colby (1948), urinary tract infections have responded particularly well, even when *E. coli* and *B. proteus* was present (Sarnoff, Freedman, and Hyman, 1946; Haines and Miceli, 1947; Narins, 1948; Lazarus and Schwarz, 1949; Carroll, Allen, and Flynn, 1950). In eighty-two patients, as well as in a study of 131 strains studied *in vitro*, Carroll and co-workers (1950) found no evidence of the development of bacterial resistance.

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The only report dealing with children exclusively is that of Bigler and Thomas (1950). They employed Gantrisin alone in the treatment of 71 children with various disorders including pneumonia, bronchitis, otitis media, tonsillitis, cervical adenitis, and urinary tract infections. According to these authors, no untoward reactions could be attributed to the use of the drug.

It is the purpose of this investigation to ascertain the dosage of Gantrisin necessary to obtain effective blood levels when administered orally, intramuscularly, intravenously, and subcutaneously to infants and children. Observations also have been made regarding toxic effects and therapeutic impressions in the children studied.

Materials and Methods

One hundred and thirty-six infants and children from the outpatient clinic and the wards of the Children's Hospital of The University of Texas Medical Branch have been studied. Of these, thirty-nine were convalescent subjects, free from renal or cardiovascular diseases, who were given a single dose of the drug and the blood levels† determined periodically. Single doses for oral administration were 0.05 gm., 0.10 gm., and 0.15 gm. per kg. Blood levels, determined as the free sulmonamide, were obtained at 3, 6, 9, and 12 hours. Single doses used intramuscularly, subcutaneously and intravenously were 0.10 gm. per kg. Blood levels were determined at 3, 6, 9, and 12 hours after intramuscular injection and at one and 4 hours after subcutaneous and intravenous routes of administration.

The remaining 97 patients, acutely ill with various infections, were given the drug periodically. Of these, 82 were given Gantrisin alone, and the others were given the drug in combination with various antibiotics. In the patients weighing less than 30 kg. the initial dose was 0.10 gm. per kg., and the maintenance dose either 0.20 gm. or 0.25 gm. per kg. per day. A few patients weighing less than 30 kg. were given 0.16 gm. per kg. per day. The daily maintenance dosage was

†The co-operation of Lloyd Sekaly of the Clinical Laboratory of the Medical Branch Hospitals is gratefully acknowledged.

administered in divided portions: orally, every 6 hours; intramuscularly, every 6 or 8 hours; subcutaneously, every 12 hours or continuously; intravenously, every 8 hours or continuously. In patients weighing more than 30 kg. the initial dose was 3.0 to 4.0 gm. with a total daily dose of 6.0 to 8.0 gm.

Blood levels, taken midway between doses, were determined in 47 of the group of 97 patients suffering from the various infections. Orally, Gantrisin was administered in 0.5 gm. tablets or as a chocolate flavored syrup containing 0.5 gm. in 5 ml. For the parenteral routes of administration, the 40 per cent diethanolamine salt solution was employed. When given intramuscularly, the 40 per cent solution was used undiluted and occasionally this concentration was given intravenously. When administered subcutaneously, the concentration was reduced to 5 per cent or even less by dilution, *e.g.*, saline or 2.5 per cent dextrose. On occasion it was added to the intravenous solutions given by the drip method. No alkalies were given concomitantly. Fluid consumption was that normally required.

Results

Oral Administration

Single Dose: The average blood levels obtained in 28 patients given three different doses orally are presented in Figure 1. In six patients, given 0.05 gm. per kg., the average blood level at 3 hours was 5.2, at 6 hours 3.2 and at 9 and 12 hours, respectively, was only 2.0 and 1.4 mg. per cent. Twelve patients given 0.10 gm. per kg. revealed average blood levels at 3 hours of 9.4, at 6 hours 6.3, at 9 hours, 3.5, and at 12 hours only 1.8 mg. per cent. Ten patients who received 0.15 gm. per kg. showed an average blood level at 3 hours of 13.7, at 6 hours 8.9, at 9 hours 4.7, and at 12 hours 3.4 mg. per cent. The individual blood level curves of the twelve patients given 0.10 gm. per kg. and the ten patients given 0.15 gm. per kg. are shown in Figures 2 and 3, respectively. In the children given the larger amounts the range of blood levels was higher throughout the period of study.

Continuous Administration: The blood levels on 30 patients receiving the drug over a period of days are shown in Figure 4. In patients receiving a maintenance dose of 0.20 gm. per kg. per day the blood levels ranged from 5.1 to 13.9 with an

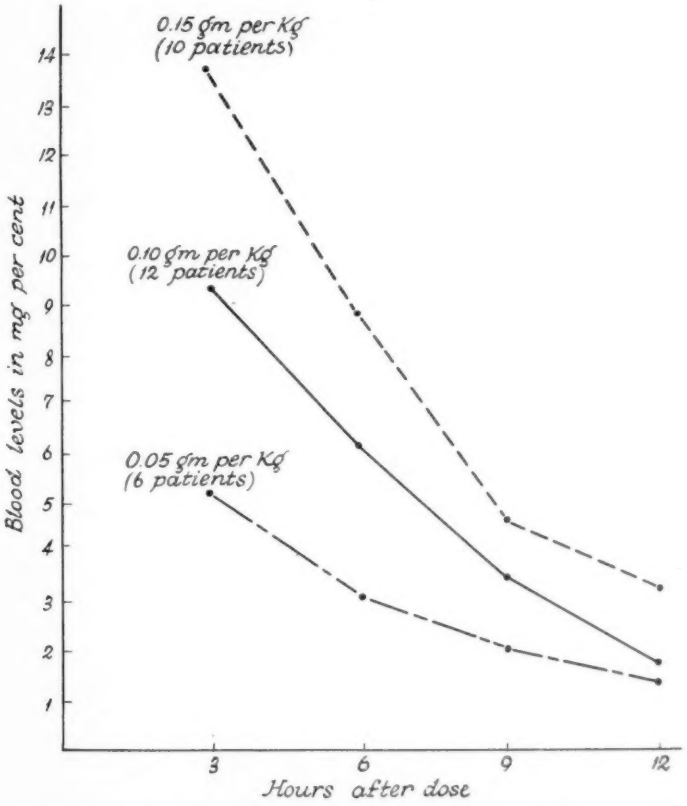

FIG. 1. Average blood levels of free sulfonamide obtained following the oral administration of 0.05, 0.10 and 0.15 gm. per kg. Gantrisin to 28 children.

average value of 8.6 mg. per cent. In seventeen patients receiving a maintenance dose of 0.25 gm. per kg. per day the blood levels ranged from 5.8 to 19.0 with an average level of

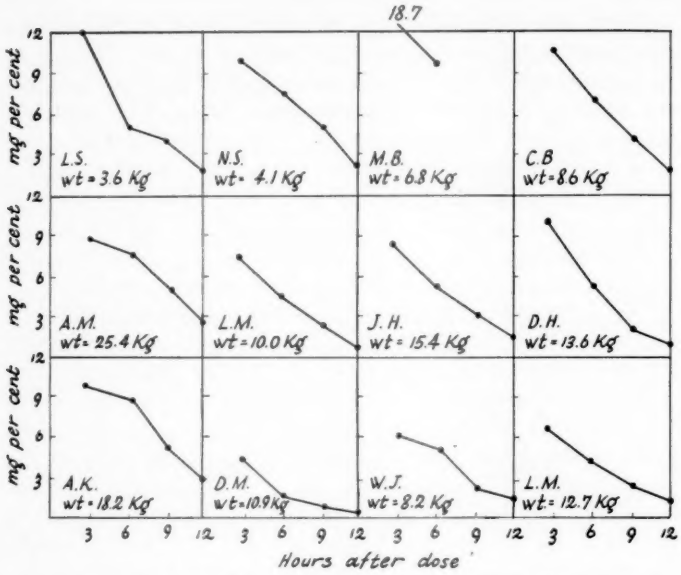
18.7
mg per cent
L.S. wt = 3.6 Kg
N.S. wt = 4.1 Kg
M.B. wt = 6.8 Kg
C.B wt = 8.6 Kg
A.M. wt = 25.4 Kg
L.M. wt = 10.0 Kg
J.H. wt = 15.4 Kg
D.H. wt = 13.6 Kg
A.K. wt = 18.2 Kg
D.M. wt = 10.9 Kg
W.J. wt = 8.2 Kg
L.M. wt = 12.7 Kg
Hours after dose

FIG. 2. Blood levels of free sulfonamide in 12 patients given 0.10 gm. per kg. Gantrisin orally as a single dose.

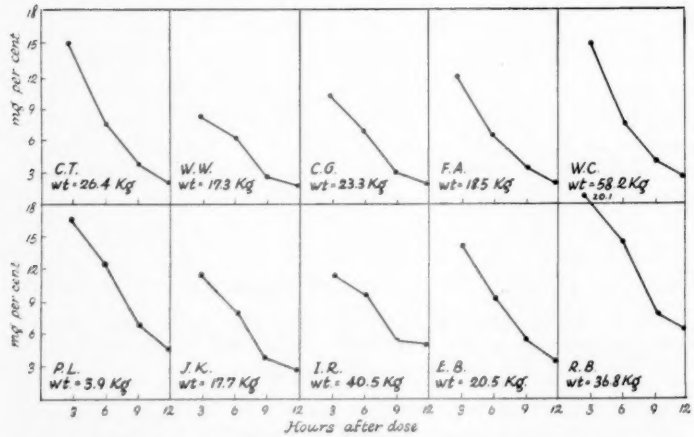
mg per cent
C.T. wt = 26.4 Kg
W.W. wt = 17.3 Kg
C.G. wt = 23.3 Kg
F.A. wt = 18.5 Kg
W.C. wt = 58.2 Kg
P.L. wt = 3.9 Kg
J.K. wt = 17.7 Kg
I.R. wt = 40.5 Kg
E.B. wt = 20.5 Kg
R.B wt = 36.8 Kg
Hours after dose

FIG. 3. Blood levels of free sulfonamide in 10 patients given 0.15 gm. per kg. Gantrisin orally as a single dose.

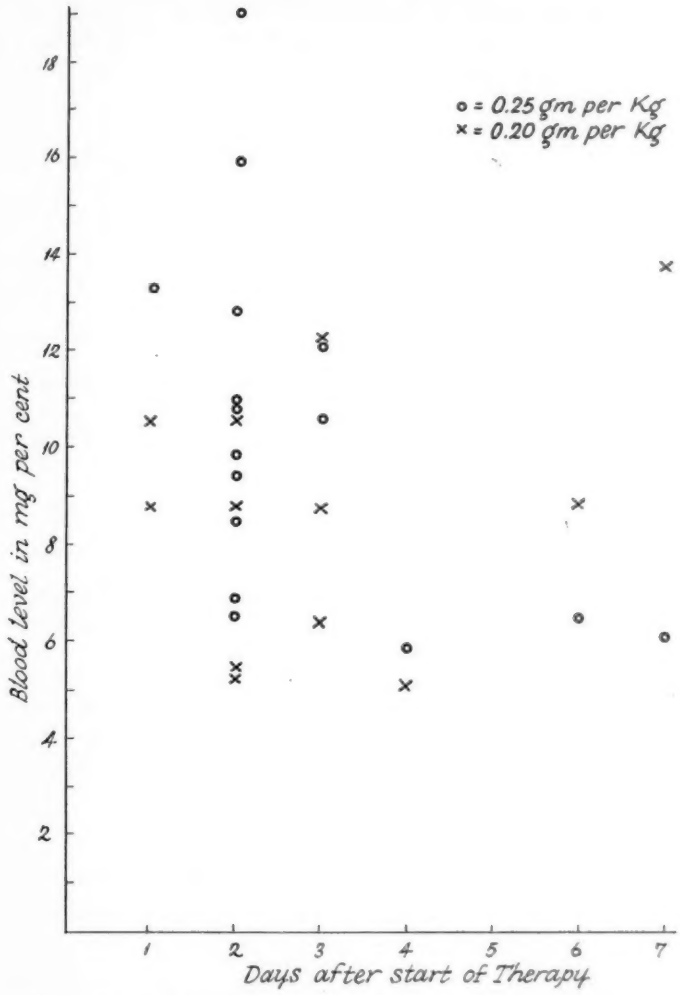

FIG. 4. Blood levels of free sulfonamide in 28 acutely ill subjects receiving 0.20 or 0.25 gm. per kg. per day, determined three hours following the previous dose after one to seven days of therapy.

10.6 mg. per cent. In two patients weighing 36.4 kg. and 34.1 kg. who were given 6.0 gm. daily the blood levels were 9.3 and 8.0 mg. per cent, respectively.

Intramuscular Administration

Single Dose: In four patients given a single dose of 0.10 gm. per kg. body weight, average blood levels at 3 hours after injection were 10.6, at 6 hours 6.8, at 9 hours 4.5, and at 12 hours 2.0 mg. per cent. These results are shown in Table I.

TABLE I

Gantrisin Blood Levels After Single Intramuscular Dose of 0.10 gm. per kg. Body Weight.

Patient	Wt. in kg.	Blood Level in mg. per cent			
		3 hr.	6 hr.	9 hr.	12 hr.
E.M.	20.0	10.9	8.0	4.0	2.5
L.L.	8.2	6.8	2.7	..	0.4
R.H.	13.2	12.2	8.6	6.0	3.5
I.R.	10.9	12.7	7.4	3.5	1.7

Continuous Administration: The blood levels for 8 patients receiving 0.2 to 0.25 gm. as the daily dosage are presented in Table II. The average blood levels when determined 3 hours after injection for five patients receiving 0.20 gm. per kg. daily in divided doses every 6 hours was 10.3 mg. per cent. Only two patients were treated with 0.25 gm. per day at 8-hour intervals and in these the blood levels were not much different.

TABLE II

Gantrisin Blood Levels During Intramuscular Therapy

Patient	Wt. in kg.	Daily Dosage gm. per kg.	Interval of Administration	Days after starting therapy	Blood Level mg. per cent
R.W.	14.1	0.20	6 hours	1	14.9
J.N.	7.7	0.20	6 hours	3	10.6
A.R.	18.1	0.20	6 hours	3	10.8
L.W.	9.1	0.20	6 hours	2	8.3
V.B.	9.5	0.20	6 hours	1	7.1
T.W.	3.6	0.25	6 hours	2	15.3
L.F.	12.7	0.25	8 hours	1	11.1
C.M.	15.9	0.25	8 hours	1	14.0

In the latter two subjects the blood levels after one hour were 15.0 and 16.5; after four hours 11.1 and 14.0; and after eight hours 3.5 and 5.2 mg. per cent, respectively.

Subcutaneous Administration

Single Dose: The blood levels in four patients who were given a single dose of 0.10 gm. per kg. body weight as a 5 per cent solution or placed in clysis fluids every 12 hours are shown in Table III. With 0.20 gm. per kg. at the end of the first 12 hours the average blood level was 10.4, and at 24 hours 7.1 mg. per cent. These results are shown in Table IV.

TABLE III

Gantrisin Blood Levels Following Single Subcutaneous Administration of 0.10 gm. per kg. Body Weight as a 5 Per Cent Solution in Distilled Water.

Patient	Wt. in kg.	Blood Level in mg. per cent	
		1 hr.	4 hrs.
M.L.*	5.4	8.8	4.5
T.C.	9.1	12.8	11.3
C.L.	8.2	15.0	14.0
R.L.	7.3	16.7	14.9

*One per cent solution used.

TABLE IV

Gantrisin Blood Levels During Subcutaneous Administration of 0.20 gm. per kg. Daily.

Patient	Wt. in kg.	Blood Level in mg. per cent	
		12 hrs.	24 hrs.
N.C.	5.9	10.1	7.6
J.S.	12.5	17.1	8.8
S.P.	10.4	4.2	4.4
E.G.	20.0	...	7.5

Intravenous Administration

Single Dose: Blood levels in three patients following a single intravenous dose of 0.1 gm. per kg. are shown in Table V. In one hour the average was 14.4 and after four hours was 8.0 mg. per cent.

TABLE V

Gantrisin Blood Levels Following Single Intravenous Dose of 0.10 gm. per kg.

Patient	Wt. in kg.	Blood Level in mg. per cent	
		1 hr.	4 hrs.
F.R.	16.4	15.0	5.7
J.W.	15.0	12.8	8.4
L.F.	13.2	15.6	10.0

Continuous Administration: Two patients who were treated with a daily dose of 0.20 gm. per kg. in divided doses every eight hours for 24 hours of therapy obtained the following blood levels: after the last dose at one hour, 14.8 mg. and 15.0; at 4 hours, 6.2 and 7.9; at 8 hours 3.5 mg. and 4.0 mg. per cent. In the one patient who was given 0.20 gm. per kg. daily in a continuous intravenous infusion after 24 hours the blood level was 8.4 mg. per cent.

Clinical Observations

Data concerning the 82 children who were given Gantrisin as the sole therapeutic agent are summarized in Table VI.

The six patients with acute tonsillitis ranged in age from one to 11 years, and five seemed to respond rapidly. The other patient improved only slightly in 48 hours, hence penicillin was started in combination with Gantrisin. Beta-hemolytic *Streptococcus* was isolated from the throat in this patient.

The largest group ranging from two months to seven years, suffered from acute otitis media. Five of these patients had an acute purulent otitis media while the remaining twenty-one had only catarrhal involvement. The average range of temperature was 101° to 102°. Of the former group, temperatures returned to normal within 24 to 48 hours, and the drainage ceased in all instances in from 2 to 7 days. In the twenty-one patients with acute catarrhal otitis media, subsidence of fever in sixteen was rapid. Five patients were considered to have a poor response. One of these patients vomited repeatedly while taking the drug, so it was discontinued and penicillin was administered. The remaining four patients were given penicillin with no especial change in their condition. In one

instance a myringotomy was necessary. The predominant organisms in the patients who responded poorly were reported in three instances as alpha-*Streptococcus*, and in one patient as gamma-*Streptococcus*. No culture was obtained in the fifth patient.

TABLE VI

Clinical Conditions Treated with Gantrisin Alone and Interpretation of Response in 82 Infants and Children.

Number of Cases	Diagnosis	Cultures	Good Response	Poor Response
6	Tonsillitis	2 β-streptococcus 4 no cultures	5	1 β-streptococcus
26	Otitis Media	9 α-streptococcus 1 β-streptococcus 1 γ-streptococcus 1 S. aureus 1 S. albus 13 no cultures	21	5 3 α-streptococcus 1 γ-streptococcus 1 no culture
10	Pharyngitis	1 S. aureus 2 S. albus 1 β-streptococcus 1 α-streptococcus 5 no cultures	10	
2	Bronchitis	1 β-streptococcus 1 no culture	2	
1	Bronchiolitis	1 S. aureus	1	
10	Bronchopneumonia	1 α-streptococcus 1 β-streptococcus 1 Neisseria 1 S. aureus 1 S. albus 5 no cultures	8	2
2	Lobar Pneumonia	1 α-streptococcus 1 no culture	2	
16	Diarrhea	6 Sh. flexneri 4 coliforms 1 Proteus rettgerii 1 Proteus morganii 2 Proteus mirabilis 1 P. aerogenoides 1 no culture	15	1 Sh. flexneri
5	Acute Skin Infections	2 S. aureus 3 no cultures	5	
4	Urinary Tract Infections	1 coliform 2 paracolon 1 mixed	4	

There were ten children with acute pharyngitis varying in age from one to seven years. On initial examination the temperatures ranged from 101° to 105°. There was disappearance

of symptoms and subsidence of fever within 24 to 48 hours in all instances hence the response to therapy was considered good.

In the two patients with acute bronchitis and one patient with acute bronchiolitis the response was very satisfactory.

Bronchopneumonia as the clinical diagnosis confirmed by X ray was present in ten instances with ages ranging from one to six years. On initial examination temperatures varied from 101° to 103°. In eight of these patients the clinical response appeared to be excellent with defervescence in twenty-four hours in most instances. In one patient who was considered to have a poor response, the pneumonia was secondary to kerosene aspiration and improvement was slow over a period of one week. In the other, a clinical diagnosis of viral pneumonitis was made.

In two patients, aged six and thirteen years with clinical and roentgenologic signs of pneumonia with lobar distribution, improvement was prompt. Temperatures returned to normal in twenty-four hours and physical findings disappeared within 2 to 3 days.

There were seventeen patients aged one month to seven years with moderately severe to severe diarrhea. Results of rectal cultures are shown in Table VI. Six patients with *Shigella* dysentery were treated. Naturally in the management of patients with diarrhea, fluid and electrolyte balance was maintained. Glucose and saline solutions were given orally or parenterally and food was withheld for about 24 hours, then small quantities of skimmed milk were given as the diarrhea subsided. The response to therapy in sixteen patients seemed very satisfactory. The fever and diarrhea subsided within 24 to 48 hours. One patient with *Shigella* dysentery responded slowly. Although the diarrhea had ceased by the sixth day of therapy, a culture on the seventh day was still positive. Rectal swab cultures in the remaining five patients with *Shigella* infections were negative within seventy-two hours.

There were five patients with skin infections. Two of these patients had severe pyoderma, one had a furunculosis, one had a secondarily infected eczema and the last had a secondarily infected intertrigo. In forty-eight to seventy-two hours there was marked improvement in all these subjects.

In four patients with the diagnosis of acute pyelonephritis, two had complicating congenital defects of the genito-urinary tract. The clinical response was considered good in all, although in one patient the urine never became sterile during three months of Gantrisin administration. This patient had a congenital defect requiring indwelling catheters.

Discussion

On the basis of studies herein reported it appears that children weighing less than 30 kg., regardless of the method of administration, Gantrisin in an initial dose of 0.10 gm. per kg. followed by a maintenance dose of 0.20 gm. per kg. daily will give blood levels of 5.0 to 14.0 mg. per cent. These levels are considered to be clinically effective for sulfonamide susceptible infections of average severity. This dosage is slightly higher than that used by Bigler and Thomas (1950). They administered amounts of the drug ranging from 0.13 gm. to 0.19 gm. per kg. daily and obtained blood levels varying from 3.0 to 15.7 mg. per cent although average blood levels ranged from 6.4 to 10.7 mg. per cent. Smaller dosages were not used extensively in our study inasmuch as the higher blood levels obtained with larger doses appeared to be well tolerated. Optimal blood levels of Gantrisin for the various infections have not been ascertained, as yet.

A few *in vitro* sensitivity studies on pathogens isolated from the urinary tract have been reported (Rodgers and Colby, 1948; Carroll, Allen, and Flynn, 1950). Carroll and co-workers (1950), for example, found that *Proteus* organisms were sensitive to concentrations of Gantrisin varying from 1.3 to 5.0 mg. per cent. *E. coli* and *Alcaligenes* were sensitive in concentrations varying from 1.3 to 10.0 mg. per cent. *Aerobacter* and paracolon organisms were sensitive in concentrations from 10.0 to 20.0 mg. per cent while *Pseudomonas* and *Streptococcus fecalis* were sensitive only in concentrations from 20.0 to 40.0 mg. per cent.

Data bearing on possible optimal blood levels of Gantrisin have come principally from *in vivo* studies on adult patients with acute infections. Svec, Rhoads, and Rohr (1950) reported that in adult subjects a daily dose of 6.0 gm. given at intervals

of 4 hours provided average blood levels of free Gantrisin (determined just prior to the next dose) of 5.8 mg. per cent orally, 6.7 mg. per cent intramuscularly, 11.7 mg. per cent intravenously. This same group of workers found an average blood level of free Gantrisin of 9.4 mg. per cent to be effective in meningococcic meningitis. Using the same total daily dose, Brockhouse and co-workers (1949) reported good results in the treatment of 54 adult patients with various bacterial infections. In their patients, blood concentrations of Gantrisin ranged from 2.7 to 17.0 mg. per cent, although 83 per cent fell between 4.0 and 11.0 mg. per cent.

Our findings revealed that single oral doses of 0.05 gm. per kg. of Gantrisin resulted in relatively low blood levels. However, single doses of 0.10 gm. per kg. whether given orally, intramuscularly, subcutaneously or intravenously produced average blood levels greater than 9.0 mg. per cent in three hours and 6.0 mg. per cent in the six hours after administration. Not unexpectedly, single doses by the subcutaneous and intravenous routes resulted in the highest blood levels. These values did not differ significantly at one hour following single doses by these two routes, but at four hours the blood levels were considerably higher following subcutaneous administration.

The oral route of administration of course is the most practical. It was noted that a daily dose of 0.20 gm. per kg. orally insured blood levels averaging 8.6 mg. per cent. From the data observed in this study, the daily dose of 0.25 gm. per kg. resulted in slightly higher average blood levels, but the therapeutic efficacy appeared to be no greater than the smaller dose of 0.20 gm. per kg.

In patients unable to take Gantrisin by mouth as well as in severe infections requiring high blood levels, continuous administration by the intramuscular, subcutaneous, and intravenous routes all appeared to be effective. Relatively high blood levels were obtained with 0.20 gm. per kg. daily, but larger doses, which occasionally may be necessary, were well tolerated. Of the parenteral methods of administration, the intramuscular route was the most convenient. However, when parenteral fluids were necessary it was very easy to add the

drug to the solutions which were being given subcutaneously or intravenously as continuous infusions. When it was felt necessary to procure high blood levels rapidly, Gantrisin was given in a dose of 0.10 gm. per kg. administered either subcutaneously in a 5 per cent solution in distilled water or intravenously as the undiluted 40 per cent diethanolamine salt. Intramuscular or intravenous administration of 0.20 gm. to 0.25 gm. per kg. in divided doses every eight hours appeared to be effective in the subjects studied. No serious reactions were encountered in the 136 infants and children studied. Two patients, however, were observed to vomit repeatedly so that the drug had to be discontinued.

Dermatitis, anorexia, nausea, vomiting, headache, fever, and vertigo have been reported (Rhoads, Svec, and Rohr, 1949, 1950; Brickhouse, Lepper, Stone, and Dowling, 1949; Thompson, 1949; Narins, 1948; Rodgers and Colby, 1948; Lazarus and Schwarz, 1949) as complications but the incidence seems to be no greater than for other sulfonamides. Eight instances of leukopenia have been cited (Thompson, 1949; Lazarus and Schwarz, 1949). Haunz, Cardy, and Graham (1950), recently reported the first case of agranulocytosis from this drug. These warnings emphasize the necessity for observing the usual precautions for patients receiving sulfonamides. No local reactions were observed following subcutaneous administration of Gantrisin solutions in concentration of 5 per cent or less or in intravenous administration of the undiluted 40 per cent diethanolamine salt solution.

Occasional slightly painful indurated areas were observed following intramuscular injection but there were not serious. One patient, however, developed a sterile abscess following careless administration of the 40 per cent diethanolamine salt solution subcutaneously instead of intramuscularly. Repeated urinalyses on 51 patients receiving the drug, revealed no evidence of hematuria. In one very dehydrated patient crystalluria was observed on the first day of therapy but without stopping the drug this disappeared on the second day and did not reappear during the treatment period. Peripheral blood counts were determined before and during therapy on forty-two patients. There was no evidence of any abnormality in the erythrocytic

or granulocytic series in this group. Bone marrow studies have not been reported in the patients with leukopenia except in the woman with agranulocytoses. The impression of the clinical staff members was that the response to Gantrisin in acute bacterial infections was satisfactory. Although control studies of similarly affected children were not attempted, it was the clinical impression of the observers that Gantrisin produced as good results as sulfadiazine when used for treatment of hospitalized and clinic patients with bacterial infections.

Conclusions

In the practice of medicine with infants and children where the difficulty of fluid administration is sometimes great and where administration of alkalinizing agents are potentially dangerous, Gantrisin offers the advantage of an effective sulfonamide of high solubility with little danger of renal complications. Determination of blood levels in 136 infants and children following various doses of Gantrisin administered by the different routes was made. These data indicate that an initial dose of 0.1 gm. per kg. and a maintenance dose of 0.20 gm. per kg. daily will produce effective blood levels in the range of 6 to 14 mg. per cent depending upon the route of administration.

The oral route is the most practical method of adminstration. In patients who are vomiting or who are seriously ill and in whom high blood levels are desirable, Gantrisin may be administered by the intramuscular, subcutaneous, or intravenous routes. Results of therapy in 82 ill patients given Gantrisin as the sole therapeutic agent on the whole were considered to be satisfactory. No serious toxic effects were observed in the children studied, nevertheless it is recommended that the usual precautions for patients receiving sulfonamides be observed.

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PROCEEDINGS OF THE TEXAS BRANCH OF THE SOCIETY OF AMERICAN BACTERIOLOGISTS

Spring (20th) Meeting, Baylor University College of Medicine, Houston, Texas, May 19, 1951

Titles

Intracellular polysaccharide of *Enterobacteriaceae*. Helen Hoyo, C. E. Lankford, and J. F. Lutteringer, Department of Bacteriology, The University of Texas, Austin, Texas.

Production of histamine from imidazole substrates by *Escherichia coli*. Joe B. Nash, Joyce K. Howard, and Paul L. Ewing, Department of Pharmacology, The University of Texas Medical Branch, Galveston, Texas.

The effect of certain antibiotics, antimalarial drugs, and amebicides on *Candida albicans*. Oleta Beck and Helen Lacy, Department of Bacteriology, The University of Texas Medical Branch, Galveston, Texas.

In vitro and clinical activity of diaphine against *Candida albicans*. Joyce K. Howard, Garth L. Jarvis, Randolph Clements, and G. A. Emerson, Departments of Pharmacology and Obstetrics, The University of Texas Medical Branch, Galveston, Texas.

Toxicity of elemental sulfur for *Brucella*. V. T. Schuhardt, L. J. Rode, Glenda Oglesby, and C. E. Lankford, Brucellosis Research Laboratory of the Clayton Foundation, The University of Texas, Austin, Texas.

Laboratory findings on the recent outbreak of influenza. Thelma D. Sullivan, James McNeese, and J. V. Irons, Bureau of Laboratories, State Health Department, Austin, Texas.

Some field and laboratory notes on *Coxiella burnetti*. Margaret Neville Norris, Clifford W. Johnson, O. L. Walker, Mary Crabbe, and J. V. Irons, Bureau of Laboratories, State Health Department, Austin, Texas.

A specific complement fixation test for poliomyelitis. Morris Pollard, Department of Preventive Medicine and Public Health, The University of Texas Medical Branch, Galveston, Texas.

Agglutinins for *Leptospira* in rats trapped in Dallas. Robert M. Pike, Robin I. Burdette, and Elias Strauss, Department of Bacteriology and Immunology, Southwestern Medical School of The University of Texas, Dallas, Texas.

Production of a specific antiserum against sickle cell anemia erythrocytes. Rose Schnieder and William C. Levin, Department of Neurology and Medicine, The University of Texas Medical Branch, Galveston, Texas.

I. M. Lewis, *Lecturer*
Dr. Rolla E. Dyer, A.B., M.D., LL.D.
Director, National Institutes of Health *(retired)*
Director of Research, Emory University

Lecture Topic: *Q Fever*

Abstracts

The Sterilization of Antigens, Antisera, and Antibiotics with Ethylene Oxide. Frank B. Engley, Jr., Department of Bacteriology and Parasitology, The University of Texas Medical Branch, Galveston.

Ethylene oxide was found to be a practical sterilizing agent for a *Salmonella typhosa* vaccine. No change in antigenicity or in specificity was noted when agglutinin absorption tests or cross-agglutination tests were carried out with closely related *Salmonella* species.

Crystalline bovine plasma albumin (Armour) and its corresponding rabbit antiserum were utilized as a sensitive quantitative precipitating system. It was observed that combinations of ethylene-oxide-treated, and untreated antigen and antibody, gave a similar equivalence zone indicating no change in specificity by the ethylene oxide treatment. In the region of antigen excess, less precipitation was observed with both the treated antigen and antiserum suggesting a change in solubility of the antigen-antibody complex.

Solutions of antibiotics (aureomycin, bacitracin, chloromycetin, neomycin and penicillin) which had been contaminated with a mixture of *S. aureus*, *E. coli* and spores of *B. globigii* were sterilized by ethylene oxide treatment without loss of antibiotic activity. When similar antibiotics on discs for assay procedures were sterilized by gaseous ethylene oxide, slight loss in activity was observed in some instances.

Some Characteristics of Microsporum Gypseum. William B. Sharp, Department of Bacteriology and Parasitology, The University of Texas Medical Branch, Galveston.

Recent evidences that *M. gypseum* infection occurs more commonly in this country than had been assumed bring a renewal of interest in the biologic characteristics of that organism. The present serologic study extends to the *M. gypseum* some observations previously reported as for other dermatophytes. Results indicate that as close a relationship does not exist between it and *M. audouini* or *M. canis* as exists between the latter two species.

Universal Serologic Reaction in Neoplasia. Leonard R. Ortega, Cora L. Gott, and E. Staten Wynne. Divisions of Clinical and Experimental Pathology, The University of Texas M. D. Anderson Hospital for Cancer Research, Houston.

The universal serologic reaction as developed by Kahn was studied in patients with neoplastic disease. Results failed to confirm the findings of Lind and Ashley that a doubtful precipitate occurs in zone II in cases of advanced malignancy. There was a general trend toward less precipitation in all three zones in sera from patients with malignant neoplastic disease, but in individual cases the variation was much too great for consideration of the universal serologic reaction as having any diagnostic value in cancer.

In general, it was noted on successive tests that there was an increase in the amount of precipitation in patients who showed clinical improvement following successful treatment and a decrease in those with metastases and poor physical conditions. Results of the investigation seem compatible with the concept of recent workers that malignant tumors cause an impairment of the immunological response mechanism. Hence reduction of the amount of precipitation with decline or death of the individual would arise from inhibition of the antibody producing mechanism, and increase with successful treatment would indicate restoration of this mechanism.

Identification of the 1951 Influenza Epidemic. G. King and M. Pollard.

From January 31, 1951, to February 28, 1951, one hundred twenty-two cases of symptomatic influenza were observed at the Student Health Clinic. Thirty-eight were studied for serological identification of the etiological agent by the hemagglutination inhibition (HI) technique on acute and convalescent serums.

Twenty-five of thirty-eight patients developed antibodies for A′influenza. The other thirteen were negative for influenza strains, A, A′, and B.

From the garglings of two patients, two hemagglutinating agents were isolated in the amniotic fluids of developing chick embryos. Material from the first passage agglutinated guinea pig rbc and amniotic material from subsequent passages agglutinated chicken rbc. HI tests with known serums identified the two newly isolated agents as A′.

In order to eliminate the possibility of another strain of virus being involved in this epidemic, six garglings from serologically negative cases were inoculated into the amniotic sac of developing chick embryos with negative results.

Effect of Age of Serum on Microbiological Response. E. Staten Wynne and Donald A. Mehl. The University of Texas M. D. Anderson Hospital for Cancer Research, Houston.

Sera from presumably fasting normal persons were tested in a basal glucose-salts medium supplemented with 0.03–0.05% Difco yeast extract. Responses were measured as optical densities.

Chief findings follow:

Six pools of sera collected from the same five volunteer donors up to 28 days prior to examination were tested simultaneously. With *Lactobacillus casei*, response decreased markedly with age of sera, while less pronounced effect occurred with two strains of *Lactobacillus arabinosus* and little or no effect with *Lactobacillus brevis* or *Micrococcus pyogenes aureus*.

Fifteen organisms were examined by a carefully standardized procedure using one large serum pool at varying times after collection. Two strains of *Streptococcus fecalis*, *Streptococcus lactis*, *Leuconostoc mesenteroides* (Texas), *Leuconostoc citrovorum*, two strains of *L. arabinosus*, and *L. casei* showed significantly lower responses to aged serum. *Leuconostoc dextranicum*, *L. mesenteroides* ATCC 8293, *Lactobacillus fermenti*, and *L. brevis* showed no significant effect of age of serum; while *M. pyogenes aureus*, *L. mesenteroides* P-60, and *Lactobacillus pentoaceticus* gave equivocal data.

No explanation for the phenomenon is offered. Atmospheric oxygen is apparently not required, and the addition of 0.05% soluble starch had no reversing action.

Studies on the Mechanism of Antagonism by *Aerobacter* Strains. Robert E. Bowling and E. Staten Wynne. State Department of Health Laboratories, Oklahoma City, Oklahoma, and Department of Experimental Pathology, The University of Texas M. D. Anderson Hospital for Cancer Research, Houston.

Fourteen *Aerobacter* strains were tested against seven common bacterial species by the "staled" agar plate technique. Antagonism in varying degrees was noted in all cases. Further work was done with four of the most antagonistic *Aerobacter* strains and four test species. Two of the latter were completely inhibited and the other two were strongly inhibited.

Evidence for a diffusible inhibitory substance could not be obtained through use of *Aerobacter* culture filtrates, cellophane bag dialysates, heat-killed suspensions, chloroform-killed suspensions, or plates seeded with the test species and streaked with *Aerobacter*. Furthermore, washed cells gave inhibition equal to that obtained with whole cultures, and 12-hour cultures were equivalent to 72-hour cultures. Inhibition was noted in plates prepared from dilutions of *Aerobacter* cultures up to 1/10,000, at which dilution all four test species were inhibited to approximately the same degree.

"Direct antagonism" (Neufeld and Kuhn, Z. Hyg. Infektionskrankh. 116:95–110, 1934) involving relatively close contact of living *Aerobacter* cells with cells of the inhibited species is felt at present to be the most feasible explanation of *Aerobacter* antagonism.

Taxonomic Position of Strains of Aerobic Sporeforming Bacilli of Importance in Antibiotic Work. Kenneth L. Burdon and Reuben D. Wende, Department of Microbiology, Baylor University College of Medicine, Houston.

Twenty-five cultures, representing strains of aerobic sporeforming bacilli currently used in the United States for the production or assay of antibiotics were subjected to a systematic study to establish more definitely their taxonomic status, and to discover peculiarities, if any, which would differentiate them from stock strains of known identity. Of particular

interest were the considerable number of cultures labelled *B. subtilis* (without qualification), since at least three distinctly different organisms have been so named by different workers in recent years. The original differential procedures and keys for identification of species in the genus *Bacillus*, recently worked out by the senior author, as well as techniques suggested by others, were used.

Cultures in the collection belonging to species other than *B. subtilis* were found not to differ significantly from known stock strains. Of 14 cultures labelled *B. subtilis* all but two had the properties of the classical Marburg strains of that species. One of the assay organisms, however (A.T.T.C. #9800), and the Bacitracin-producing strains were shown to be typical examples of the so-called "Ford type" of *B. subtilis*. The latter is not officially recognized as a distinct variety by American taxonomists, despite earlier descriptions by the senior author, Lamanna, and others, and despite the fact that in England it has been designated as a separate species under the name *B. licheniformis*. Differences between the Marburg and the "Ford" strains, including points of practical differentiation not previously described, were found to be clear-cut, and the universal recognition of the "Ford type" as a separate species was urged. At the same time it was pointed out that reference to these bacilli as the "Ford" strain is not really appropriate, since Ford's name has long been associated with certain well-known cultures of the typical Marburg *B. subtilis* as well, *e.g.*, A.T.T.C. #102.

It was noted also that one of the antibiotics (Ayfivin) isolated by the English bacteriologists from certain strains of *B. licheniformis* is apparently identical with Bacitracin.

TOXICITY OF VARIOUS CORTISONE PREPARATIONS ON EMBRYONIC CHICK HEART, SPLEEN, AND SPINAL CORD IN TISSUE CULTURE*

BELLE RUSKIN, CHARLES M. POMERAT, AND ARTHUR RUSKIN

Well authenticated effects of cortisone include limitation of inflammatory (Dougherty and Schneebeli, 1950; Dougherty, 1951; Michael and Whorton, 1951) and allergic (Seifter *et al.*, 1950; Tillotson, 1951) exudation, retardation of granulation tissue and wound healing (Blunt *et al.*, 1949; Ragan *et al.*, 1949, 1950), antihyaluronidase activity (Opsahl, 1949; Seifter *et al.*, 1949), and reversal of the acute manifestations of diseases affecting the widespread mesenchymal tissues (Hench *et al.*, 1950). There are also indications that cortisone actions are not mediated either neurogenically or hormonally, but are direct effects upon the tissues involved (Baker, 1951; Shapiro *et al.*, 1951). The tissue culture method is ideal from the standpoint of determining these direct effects on genetically homogeneous organs.

Material and Method

To test this hypothesis, we utilized the hanging-drop technique used for the assay of numerous other drugs in this laboratory (Painter *et al.*, 1949; Everett *et al.*, 1951) for the determination of cortisone inhibition of outgrowth from various embryonic tissues. The 5- to 7-day-old heart, 17-day-old spleen, and 9-day-old spinal cord of the chick embryo were chosen because of the rapid growth of tissues of those ages. These organs also served as representatives of muscular, reticuloendothelial-hemic, and nervous tissues, respectively. Four to eight preparations served as controls for each set of drug experiments. Each dilution of a drug was tested in quadruplicate. A total of more than 700 cultures was used in these determinations.

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The difficulties attendant to aqueous solubility of salts of cortisone were resolved as follows:

Cortisone acetate, 50 mg., was dissolved in 15 ml. of 95% alcohol. To this was added 5.5 ml. of Tween-80. After thorough mixing with the magnetic mixer, the alcohol was evaporated off on a steam bath. The solution was then made up, while mixing, to 50 ml. by adding, slowly, warm distilled water. The final solution, containing 1 mg. of cortisone/ml. of 11% Tween in water, was autoclaved for 20 min. at atmospheric pressure.

The following final concentrations of this solution, in terms of gamma/ml. of cortisone acetate and % of Tween-80, respectively, were then used with hanging-drop cultures: from 150 and 1.65, serially diluted, down to 0.59 and 0.0065. The following final concentrations of Tween-80 were also used with the hanging-drop cultures as secondary controls: from 1.65%, serially diluted, down to 0.1%.

Cortisone acetate, 8 mg., was dissolved in a sterile container in 4.8 ml. of acetone. Then 15.2 ml. of sterile distilled water was added, producing a solution of 0.4 mg. of cortisone/ml. of 24% acetone in water. Non-contaminated cultures were thus obtained without risk of acetone loss due to heat sterilization.

The following final dilutions of this solution, in terms of gamma/ml. of cortisone acetate and % of acetone, respectively, were then used with the hanging-drop cultures: 200 and 12, 100 and 6, and 50 and 3. Secondary acetone controls' final dilutions were 12, 6, and 3%. It was not possible to prepare stable concentrations of cortisone containing more than 400 gamma/ml. with this technique.

Cortisone sulphate was dissolved in distilled water and Seitz-filtered, to produce a solution of 30 mg. of cortisone sulphate/ml. of water.

The following final concentrations of this solution, in terms of gamma/ml. of cortisone sulphate, were then used with hanging-drop cultures: 15,000, diluted down to 625.

Cortisone tricarballylate† was used in the following final dilutions in gamma/ml.: 7,000, serially diluted down to 110.

†Kindly supplied by Merck and Co., in ampoules containing 14 mg. per ml. of water, made up to pH 7.5 with $NaHCO_3$.

The drug preparations, diluted in serial concentrations in Gey's solution, were mixed with an equal volume of embryonic extract (8-day chick embryos). One drop (from a 27-gauge needle) of the mixture was placed on a cover slide, then the tissue fragment and an equal drop of plasma were added and thoroughly mixed. The final drug dilution was thus 1:4. To obtain 1:2 final dilution, the drug was dissolved equally in both the Gey's solution and embryonic extract, as well as in the Gey's solution and plasma. The controls contained the same materials with the exclusion of cortisone.

The cultures, controls and those containing the test drugs, were read after incubation at 37° C., at 24 hours for spleen, and 48 hours for heart and cord. These time intervals were chosen because outgrowth at that time was particularly vigorous. The length and density of fiber outgrowth or cellular migration from the explants was graded 0 (total inhibition), +, + +, + + +, + + + + (maximum outgrowth) (C. M. Pomerat, 1951).

The minimum inhibitory dose (M.I.D.) lies between total inhibition (0) and slight outgrowth (+), as reported in the tables, while the least injurious dose (L.I.D.) is to be found between concentrations producing slight injury (usually + + +) and outgrowth resembling controls (usually + + + +). Titrations were carried out to what was considered a range of concentrations yielding reproducible data with this method.

Preparations in the range of M.I.D. and L.I.D. were fixed and stained to produce permanent records and photographic evidence of results.

Results

Tables I, II, and III summarize the results obtained with cultures of heart, spleen, and spinal cord, respectively.

Cortisone acetate in Tween-80. The least injurious dose of cortisone acetate in Tween-80 for outgrowth of heart or spleen explants was 4.7 gamma/ml. At 150.0 gamma/ml. and higher dosage there was total inhibition of outgrowth. The M.I.D. and L.I.D. for spinal cord were practically the same—150 and 9.4 gamma/ml. Since the Tween-80 concentration in the L.I.D. of the cortisone acetate solution was close to the Tween's

own L.I.D. (0.1 to 0.2%), the interpretation of cortisone acetate activity in this solution is highly questionable.

TABLE I

Minimal Concentrations of Various Agents Producing Total Inhibition (M.I.D.), Subtotal Inhibition, Least Injury (L.I.D.), and Maximal Dosage Producing No Inhibition on Heart Explants.

	M.I.D.		L.I.D.	
Agent	Total Inhibition	Slight Outgrowth	Slight Injury	Like Control
Cortisone acetate (gamma/ml.) in (Tween-80)	150.0 (1.65%)	75.0 (0.83%)	4.7 (0.05%)	2.35 (0.025%)
Tween-80	1.45%	10.83%	0.21%	0.10%
Cortisone acetate (gamma/ml.) in (Acetone)	.... not obtainable	200 (12%)	100 (6%)	50 (3%)
Acetone	50.0%	25.0%	12.5%	< 12.5%
Cortisone SO_4 (gamma/ml.)	15,000	7,500	5,000	3,750
Cortisone Tricarballylate (gamma/ml.)	7,000	3,500	875	583

Cortisone acetate in acetone. More concentrated aqueous solutions of cortisone acetate were obtained in acetone solution. Acetone itself was inhibitory in only strong concentrations (25 to 50%). Cortisone acetate in acetone was obviously less inhibitory than in Tween-80 as shown in the various tables. The L.I.D. for heart explants was 100 gamma/ml., for spleen fragments—200 gamma/ml., while the value for the spinal cord was not reached even with the maximum attainable concentrations of cortisone acetate in acetone and water. Thus, it was not possible to prepare a concentration of cortisone acetate of sufficient strength to inhibit the cord outgrowth without using the acetone solvent at a toxic level. The acetone content of the inhibitory cortisone acetate doses was below (12%) but near the injurious dosage of acetone itself (25%).

Cortisone sulphate. This compound, quite soluble in water, showed the least inhibitory effects of any of the cortisone preparations used in this study. The L.I.D. and M.I.D., equivalent for the three types of explants, were, respectively, 5,000

and 15,000 gamma/ml. Unfortunately, this soluble salt has also been found to have but slight physiological effects (Gibson, 1951).

TABLE II

Minimal Concentrations of Various Agents Producing Total Inhibition (M.I.D.), Subtotal Inhibition, Least Injury (L.I.D.), and Maximal Dosage Producing No Inhibition on Spleen Explants.

Agent	M.I.D. Total Inhibition	M.I.D. Slight Outgrowth	L.I.D. Slight Injury	L.I.D. Like Control
Cortisone acetate (gamma/ml.) in (Tween-80)	150.0 (1.65%)	75.0 (0.83%)	4.7 (0.05%)	2.35 (0.025%)
Tween-80	1.65%	0.83%	0.20%	0.10%
Cortisone acetate (gamma/ml.) in (Acetone)	not obtainable	not obtainable	200 (12%)	100 (6%)
Acetone	50.0%	25.0%	12.5%	< 12.5%
Cortisone SO_4 (gamma/ml.)	15,000	7,500	5,000	3,750
Cortisone Tricarballylate (gamma/ml.)	7,000	3,500	875	583

Cortisone tricarballylate. Some variations in results were obtained in an effort to define the toxicity of this compound upon heart, spleen, and spinal cord explants. The L.I.D.'s for these three organs were, respectively, 875, 875, and 875; the M.I.D.'s—7,000, 7,000, and 3,500 gamma/ml. These variations are considered to be within the limits of experimental error of this method. Controls with solutions of sodium bicarbonate at a pH of 7.5 produced outgrowth resembling that of untreated cultures.

Discussion

In general, the inhibitory effects of cortisone preparations upon the outgrowth from explants of heart, spleen, and spinal cord in doses corresponding to those employed clinically were very slight. This is generally true for other drugs studied with this method (*cf.* Painter, Pomerat, and Ezell, 1949).

Cortisone concentrations comparable to those maximally attained in the blood plasma from therapeutic doses (*e.g.*, 100 mg./4,000 ml. or 25 gamma/ml.) were noninhibitory in tissue culture maintained for short-term observations. This comparison is, of course, subject to the usual limitations of *in vitro* versus *in vivo* contrasts.

TABLE III

Minimal Concentrations of Various Agents Producing Total Inhibition (M.I.D.), Subtotal Inhibition, Least Injury (L.I.D.), and Maximal Dosage Producing No Inhibition on Cord Explants.

	M.I.D.		L.I.D.	
Agent	Total Inhibition	Slight Outgrowth	Slight Injury	Like Control
Cortisone acetate (gamma/ml.) in (Tween-80)	150.0 (1.65%)	75.0 (0.83%)	9.4 (0.10%)	4.7 (0.02%)
Tween-80	1.65%	0.83%	0.20%	0.10%
Cortisone acetate (gamma/ml.) in (Acetone)	(More concentrated aqueous solutions unobtainable)			200 (12.5%)
Acetone	50.0%	25.0%	12.5%	< 12.5%
Cortisone SO_4 (gamma/ml.)	15,000	7,500	5,000	3,750
Cortisone Tricarballylate (gamma/ml.)	3,500	1,750	875	583

Various adrenal steroids, including cortisone, injected directly, inhibit the growth of, or are lethal to, chick embryos (Karnofsky *et al.*, 1950). Reversible inhibition by DOCA of beating in heart fragments in tissue cultures, is also aided by cortisone in concentrations (25 to 166 gamma/ml.) comparable to those used by us (Cornman, 1950). Selective damage to fibroblasts occurs both *in vitro* (Cornman, 1950) and *in vivo* (Schneebeli, 1950) from cortisone.

In the interpretations of the results obtained with the use of cortisone acetate dissolved in Tween-80, we cannot separate the inhibitory effects of the solvent from those of the cortisone salt. The same criticism applies to some extent to the use of acetone as a solvent for cortisone acetate. However, since acetone is relatively nontoxic to the tissue cultures as

compared with Tween-80, cortisone acetate in acetone showed high L.I.D.'s of (100 to 200 gamma/ml.), which exceed the maximal plasma concentrations *in vivo* at least 4 to 8 times. The M.I.D.'s of cortisone acetate in aqueous acetone solution could not be determined because they exceeded the limits of its solubility in water and acetone.

Cortisone sulphate was the least injurious to hanging-drop cultures of the three cortisone preparations used in this investigation. The huge L.I.D.'s and M.I.D.'s are consonant with the relative therapeutic ineffectiveness of this compound of cortisone (Gibson, 1951).

The soluble tricarballylate ester of cortisone showed inhibitory effects on embryonic heart, spleen, and spinal cord intermediate between those of the clinically potent cortisone acetate and the cortisone sulphate, which is relatively inactive both *in vivo* and *in vitro*. From our data, it is unlikely that the sodium bicarbonate content of the ampoules of this compound influenced its effect upon the tissue cultures. The L.I.D.'s of cortisone tricarballylate were approximately 30 to 40 times the maximum expected plasma concentrations from therapeutic doses of cortisone acetate. The therapeutic effectiveness of cortisone tricarballylate is not inconsiderable, at least in experimental animals (Gibson, 1951).

It is necessary to emphasize that the effects upon the three types of tissues tested were accomplished in each instance with approximately the same concentrations of cortisone preparations. The L.I.D.'s for spinal cord explants, however, were somewhat higher than for other explants, when cortisone acetate in Tween-80 was employed. This observation remains unexplained.

It is impossible, at present, to relate the increased concentration of hydroxyproline in the cortisone-treated developing chick embryo (Roberts *et al.*, 1951), the inhibition of chondroitin sulphate synthesis by cortisone (Layton, 1951), or the enzymatic effects, now under study (Ruskin *et al.*, 1951), of cortisone, to its inhibition of outgrowth of tissue explants *in vitro*. Sulfhydryl enzyme inhibition may be one mechanism for the cortisone tissue effect (Anderson *et al.*, 1951).

Summary

Using more than 700 hanging-drop tissue culture preparations of chick embryo heart, spleen, and cord explants, the minimal injurious doses for total inhibition of outgrowth (M.I.D.'s) and the least injurious doses (L.I.D.'s) were determined for cortisone acetate, cortisone sulphate, and cortisone tricarballylate.

The low solubility of cortisone acetate made it impossible to separate the injury due to the solvent when Tween-80 was employed, but in the presence of acetone this cortisone preparation had an L.I.D., in gamma per ml. of medium, of between 100 to 50 for heart, 200 to 100 for spleen, while for cord the value was greater than 200. It was not possible to prepare sufficiently strong concentrations of cortisone acetate in 24% acetone to determine M.I.D. values.

Cortisone sulphate showed an M.I.D. of between 7,500 and 15,000 gamma/ml. and an L.I.D. between 3,750 and 5,000 gamma/ml. for all three kinds of tissues tested.

Cortisone tricarballylate had the following M.I.D. and L.I.D. values, respectively, in gamma/ml. of medium: for heart—3,500–7,000 and 583–875; for spleen—3,500–7,000 and 583–875; and for cord—1,750–3,500 and 583–875.

While the three cortisone preparations produced inhibitory effects which paralleled one another when tested against the three species of tissues, heart explants appeared to be somewhat more sensitive, while spinal cord and spleen cultures were slightly more resistant to injury.

Cortisone inhibition of tissue explant outgrowth may be compared to its retardation of granulation tissue formation *in vivo*. The *in vitro* inhibitory activity is slight and in the following order of decreasing effect: cortisone acetate, cortisone tricarballylate, and cortisone sulphate. This order may have *in vivo* parallels.

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RELATIONSHIP OF M. GYPSEUM WITH OTHER DERMATOPHYTES*

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Microsporum gypseum was recently reported (Sharp and Wegner, 1950) to have occurred repeatedly in Galveston, Texas, as causative agent of scalp ringworm. Correspondence tells of unreported incidence elsewhere in the nation, supporting the suggestion of more extensive incidence than has been assumed. While by no means as common as M. audouini or M. canis, the M. gypseum is not negligible as a cause of scalp ringworm in children.

A consequent growing interest in this organism among mycologists has seemed to warrant further investigation of its characteristics. Emmons (1931) among others has described morphologic and cultural features. This report bears more particularly on the biologic relationship with other dermatophytes.

Spindle shaped macroconidia in culture and other less conspicuous items of morphology place the organism in the genus Microsporum, alongside the familiar M. audouini and M. canis (Conant, 1937). Microscopy of the infected hair shaft often fails to reveal the ectothrix mosaic of small spores from which the genus derives its name, but this characteristic sheath is said to establish itself eventually and to fluoresce under Wood light (Lewis and Hopper, 1943). The three species exhibit close similarities in pathogenesis as well. Infection by M. gypseum as by the other two is one of childhood, and apart from a frequently greater inflammatory reaction it leads to similarly appearing lesions.

On the other hand, it might be said that this generic bracketing with M. audouini and M. canis is due less to a general resemblance than to the presently accepted taxonomic significance of such features as are similar. M. gypseum and they present also some marked dissimilarities. Colony appearance was formerly given weight in the differentiation of dermatophytes, and its colony no more resembles theirs than it

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does the colonies of Trichophyton or Epidermophyton species. Its morphology in tissue is distinctly different except in the stage mentioned. There is the difference cited in inflammatory reaction, once considered taxonomically significant.

Lewis and Hopper (1943) mentioned a sometimes striking morphologic resemblance to T. schoenleini in the earlier invasive stage. The spores might occur in rosaries or in no linear arrangement but do not then occur in an ectothrix sheath as do those of M. audouini and M. canis. Wilson and Plunkett (1951) find all strains recovered by them to be essentially endothrix, never more than very thinly ectothrix and not at all fluorescent. Infected hairs under Wood light are listed by Conant *et al.* (1944) as having a light greenish color instead of the bright, clear fluorescence that M. audouini and M. canis do, those infected by T. schoenleini having dull greenish.

Character of the lesion produced differs in frequently having a greater suppurative reaction. This tendency was conspicuous in the cases observed. In contrast with the milder erythema, scaling and itching of M. audouini and usually of M. canis infections, half in the M. gypseum series presented pustular discharge (Sharp and Wegner, 1950). Yellow crusted scales attached to the scalp and there was at times secondary infection involving regional lymph nodes. Lesions with the most intense inflammatory reaction were kerionic, boggy and abscess-like.

Suppurative ringworms have been known as Tinea favosa, for which Conant *et al.* (1944) list T. schoenleini, T. violaceum, or M. gypseum as causative agent. In a former classification the property was regarded as sufficient to bracket these organisms together generically. They were placed in a genus *Achorion*, defined largely by the highly inflammatory lesion produced and by infectivity for lower animals as well as man (Dodge, 1935). The distinction is only relative, since a more or less angry inflammation develops occasionally in M. canis infections (Steves and Lynch, 1947).

The point being raised is that M. gypseum shows greater resemblance to certain of the Trichophyta than do M. audouini or M. canis. This leads one to ask just how strong the biologic

relationship is that differentiates it and those other two species into the one genus.

The study at hand has to do with serologic relationship between M. gypseum and the other two. The close interrelationship between M. audouini and M. canis is indicated not only by the morphologic and other resemblances cited, but also by antigenic constitution. It has been shown that the two fail to cross-precipitate with Trichophyton or Epidermophyton strains to nearly as high titer as they do the one with the other, or even to as high titer as those others do among themselves (Sharp, 1945).

The present study set out to find whether M. gypseum also belonged to that serologic group. Cross-precipitations were made between several strains of M. gypseum and a few strains each of the other two species of Microsporum, and also between these M. gypseum strains and a few strains of other dermatophyte genera. Protein extracts of the fungus tissue were employed as antigen and the serum of rabbits immunized with such extract as antibody, the reagents being prepared in a manner previously reported (Sharp, 1941).

Representative reactions in this cross-precipitation study are shown in Table I. Results are expressed simply by titer rather than the index earlier employed, which included precipitate volume as well. In that combined index reading the differences between high titer and low titer reactions would have shown as more pronounced, but with the same distributions of each through the series.

It will be seen that the several strains of M. gypseum form a practically homogeneous group among themselves. The immunizing strain nearly always precipitates to somewhat higher titer than other strains, as also had been observed with other dermatophytes (Sharp, 1945). Reaction between strain and strain reached a titer usually of 2,000 and nearly always of 1,000.

Similar approximation is not suggested between M. gypseum and either of the other two species of Microsporum, the cross-precipitations reaching a titer of 1,000 only rarely and most commonly of from zero to 250. Similar titers to these were

noted also in the cross-precipitations between this organism and various species of Trichophyton or Epidermophyton.

TABLE I

M. gypseum Cross-Precipitations with Strains of the Same Species, of Other Microsporum Species, and of Still Other Dermatophytes.

Antigens of	Serums Immunized to M. Gypseum strain #1	strain #2	strain #3	strain #4	strain #5
M. gypseum					
strain #1.....	4000	2000	2000	500	1000
strain #2.....	2000	4000	2000	2000	2000
strain #3.....	2000	2000	2000	1000	1000
strain #4.....	2000	2000	1000	2000	1000
strain #5.....	1000	2000	2000	2000	4000
M. canis					
strain #1.....	125	250	250	0?*	0?
strain #2.....	500	1000	250	125	500
strain #3.....	500	500	500	250	250
M. audouini					
strain #1.....	500	1000	500	125	500
strain #2.....	250	250	250	0?	125
strain #3.....	125	125	125	0?	0?
T. mentagrophytes					
strain #1.....	500	2000	500	250	500
strain #2.....	125	1000	500	250	500
T. rubrum					
strain #1.....	250	500	1000	250	250
strain #2.....	125	125	125	125	125
T. epilans					
strain #1.....	0?	250	0?	0?	0?
strain #2.....	250	250	250	125	125
E. floccosum					
strain #1.....	125	500	250	0?	125
strain #2.....	250	250	500	250	1000

*The symbol "0?" indicates no precipitation in 1:125 dilution, the lowest set up.

An occasional reaction of rather high titer was obtained between the serum immune to strain #2 M. gypseum and antigens from other Microsporum species, but much the same occurred between it and antigen from one of the Trichophyton species. These readings may better be interpreted farther on in the light of tests with absorbed serums.

The serologic relationship between M. gypseum and these other species of Microsporum was further checked by precipitin absorption. By employment of an insoluble antigenic

sediment it was possible to absorb out antibody essentially as in agglutinin absorptions.

A sediment always forms in the concentrated antigen stock on standing, which in ordinary precipitin work has to be separated out and discarded to afford clear test solutions. This sediment has been found to share the antigenic property with the soluble matter. Animals may be immunized with washed suspensions of it, though thick suspensions and numerous inoculations are required. As just mentioned, it proves capable of absorbing out antibody.

For precipitin absorption work the sediment was separated off and washed by centrifuge and dried in vacuum or by standing a few days in the refrigerator. This solid absorbing matter was thickly suspended in the undiluted serum and incubated, after which it was centrifuged out for discard. When removed from the serum it took specific antibody with it.

Antibody absorption has failed in my hands to afford the sharp distinctions among molds that it does among bacteria, a difficulty noted long ago by others (Link and Wilcox, 1933). An organism's antigen tends to absorb out from another's antiserum such antibody as is shared by itself and to leave behind other of the immunizing organism's antibodies, but it does so less cleanly than bacterial antigens do.

The absorbing antigen of one dermatophyte draws somewhat irregularly on the antibody to another, though it absorbs out most readily that antibody of highest specificity to itself. An antigen might altogether remove the antibody specific to it only after repeated absorptions, and too many absorptions might remove also some of the nonspecific antibody. Acceptance of an end-point has had to depend on personal judgment.

Table II shows the relative power of M. gypseum, of M. audouini and of M. canis to absorb out antibody to the remaining two species. Unabsorbed antiserum immune to M. audouini or M. canis precipitated in high degree with the antigens of either, and practically all of this antibody was absorbed out by the antigen of either organism. This gives the same indication of close interrelationship between the two as do the quantitative precipitations.

Unabsorbed antiserum immune to M. gypseum precipitated in high degree with antigens of M. gypseum strains and in considerable degree with that of M. audouini, this being the antiserum most highly precipitative for heterologous organisms. After absorption by M. gypseum, either immunizing or another

TABLE II

Relative Ability of Microsporum Species to Absorb Out Antibody to Other Species of Microsporum.

Antiserum immune to strain	After absorption by strain	Precipitin reaction (1:125) with antigen of M. audouini strain #1	M. canis strain #1	M. gypseum strain #2	M. gypseum strain #4
M. audouini strain #1	None	+++	+++		
	M. audouini strain #1	tr.	0		
	M. canis strain #1	+	tr.		
M. canis strain #1	None	+++	+++		
	M. canis strain #1	tr.	tr.		
	M. canis strain #2	+	tr.		
	M. audouini strain #1	tr.	tr.		
M. gypseum strain #2	None	++		++++	++++
	M. gypseum strain #2	0		0	0
	M. gypseum strain #4	0		0	0
	M. audouini strain #1	tr.		++++	++++

strain, this antiserum no longer reacted with M. gypseum or M. audouini. When absorbed by M. audouini, on the other hand, it lost none of its precipitative power for strains of M. gypseum though it did lose that for M. audouini. This would indicate that the M. gypseum strains did not share the M. audouini-M. canis antigen.

One of the M. gypseum strains selected for this experiment was strain #2 whose antiserum cross-precipitated more highly than others with M. audouini or M. canis. In view of the absorption results, it is presumed that this was due not to

identical antigen but to an exceptionally high general reactivity of that serum. Its reactivity also with one of the Trichophyton species has been remarked on.

In the course of this serologic experimentation a toxic property of M. gypseum attracted my attention. It had previously been observed with dermatophytes and certain other molds that in some cases the protein extracts used as antigen were toxic for guinea pigs and rabbits. It now develops that M. gypseum extracts show greater lethal effect than those of other dermatophytes so far worked with, these others including M. audouini, M. canis, T. mentagrophytes, T. rubrum, T. epilans, and E. floccosum. Death in rabbits was caused only occasionally in the case of M. audouini or M. canis, somewhat more frequently in the case of certain Trichophyton species, and more frequently still in the case of M. gypseum.

Whether any significance attaches to this property remains to be seen. I have speculated on whether the higher degree of toxicity might correlate with the greater inflammatory reaction of M. gypseum ringworms, but what little has been learned about the distribution among the Trichophyton species does not lead to any such presumption.

The toxic effect has become evident as a rule in the course of immunizations. Animals tolerate the earlier inoculations without ill effect, but after one of those later in the series they sicken, decline rapidly and within a day or so die. The manifestations appear to have an allergic basis, though not at all like the anaphylaxis which may also be encountered. Tissues examined post mortem† have revealed only acute degenerations and not the fibrous changes to be expected from a cumulative toxic effect of all inoculations given.

Post mortem manifestations followed a generally simliar pattern and included extensive edema, conspicuous hemorrhages subcutaneously, on serous surfaces and elsewhere, and granular degeneration in viscera. The most striking tissue changes were found to occur in the liver, in an illustrative instance being as follows. The parenchyma contained scattered areas of extensive degeneration and even necrosis, with involvement largely of midzonal and peripheral portions of the

†Through the courtesy of Dr. Paul Brindley.

lobules. Nuclei of the liver cord cells in many instances disappeared and the cells fused together. The remaining liver cells contained finely vacuolated cytoplasm.

A majority of all animals injected with M. gypseum extract failed to live through the usual immunizing series of injections. Of six strains of the organism employed, two were given to a single animal each without ill effect, three killed the first rabbit but were given successfully to a second, and one killed all of three rabbits inoculated with it. The ill effects followed the third inoculation in two instances, the fourth in one, the fifth in two and the sixth in one.

The toxic effect appeared to be due undoubtedly to the fungus extracts given. All of numerous uninoculated animals that occupied nearby cages and had the same animal care remained healthy.

Conclusions

M. gypseum does not have the close serologic relationship with M. audouini and M. canis that these latter two have with one another. It is related to them more distantly, as it is to species of other dermatophyte genera. An endotoxic product present in various dermatophytes is of notably high content in M. gypseum and low in either of the other Microsporum species.

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DEVELOPMENT OF THE ANTERIOR CEREBRAL NERVE PLEXUS*

JOHN G. SINCLAIR

A ganglionated plexus was first seen in the dura of the Porpoise and tentatively assigned to the terminal nerve complex by Sinclair (1950–51). It was later studied in a human sixth month fetus, in a premature human infant, and in an infant of four months. In the latter it was apparent that the ganglia and nerves found in the falx were separate from the terminal and olfactory complex. The ganglia contain more than 850 nerve cells on each side and their form and size indicate that they may contain both afferent and postganglionic types. It was suggested (Sinclair, 1951) that they were connected rather with the anterior ethmoidal branch of the trigeminal nerve and supplied the vascular plexus of the falx and the field of the anterior cerebral artery.

The present report attempts to carry the history of this plexus back in several series of human embryos from our own collection. The oldest fetus available in section was 43. mm. in length. Figure 1 shows the ganglia of this plexus between the olfactory lobes dorsal to the crista galli. The connective tissue between the ganglia becomes the falx cerebri. This figure may be compared with figure 4 of Sinclair (1951) taken from a four-month infant at the same level and plane. The ganglia are seen to be bilaterally placed and lack the obvious cross connections which are seen in later stages. They show no connections with the olfactory lobes. Nerve fibers connecting the ganglia are poorly developed.

An embryo of 20 mm. in sagittal section (Fig. 2) shows a long cluster of basophil cells just dorsal to the ethmoid plate and inside the frontal plate. These plates at 20 mm. show neither definitive cartilage nor bone. The ganglion cell aggregation is directed upward between the olfactory lobes toward the very vascular plexus shown in figure 3. Ventral to the

*From the Department of Anatomy, The University of Texas Medical Branch, Galveston. Edgar Grünwaldt stained the 20 mm. series in silver. N. Monger of the Tissue Culture Laboratory photographed the slides. Received for publication July 25, 1951.

ethmoid is a fascicle of olfactory nerve. The section is silver impregnated by a Hortega method and shows, among the diffuse brown olfactory fibers, very sharply defined black fibers of the terminal nerve (Fig. 4). None of these fibers is to be found among the ganglionic cluster above the ethmoid, indicating that the cells are immature.

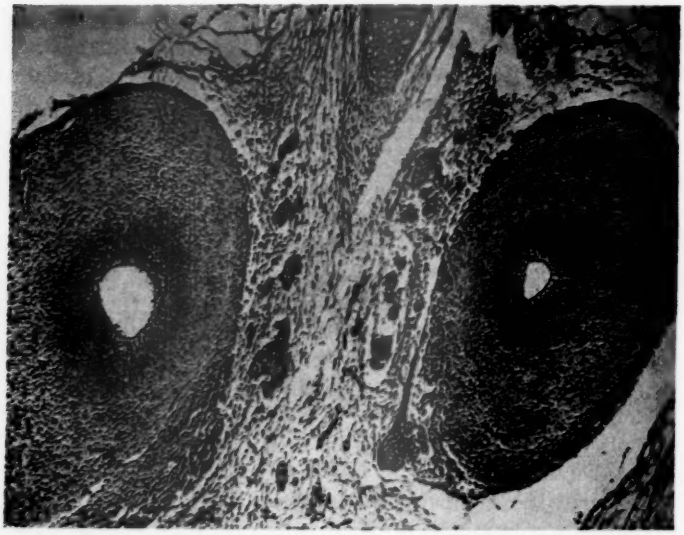

FIG. 1. Transverse section of a 43 mm. human fetus through the olfactory lobes and crista galli showing the ganglionated plexus bordering the incipient falx cerebri. Longitudinal connections are poorly developed. Transverse connections are missing.

An 18 mm. series (Fig. 5) shows the same plexus in a cross section of the head. The two streams of cells are more widely separated on either border of the ethmoid plate. When the ethmoid septum of the same embryo is traced ventrally some of these cell clusters are seen to follow the course of the vomeronasal nerve to the vomeronasal organ.

A 12 mm. embryo was investigated and the same elongated cell cluster was seen between the nasal vesicle and the base of the forebrain on either side of the head (Fig. 6). The two groups at this stage are very widely separated. They are in-

timately in contact with a vascular plexus. Olfactory fibers between brain and nasal vesicle dip transversely under this column. The cells are much less differentiated than those of the 18 mm. stage. Without the continuous history into discrete ganglia it would not be possible to determine that they are nerve cells. They differentiate along the course of the ophthalmic division of the trigeminal nerve and at least two such localizations may be seen at the 12 mm. stage.

FIG. 2. Sagittal section of the frontal-ethmoidal angle of the head of a 20 mm. human embryo with silver impregnation. The planes of the undifferentiated cranium are indicated. Olfactory nerve filaments lie outside to the left. The nerve cell clusters are directed from the ethmoid upward between olfactory lobes.

An attempt was made to find equivalent cells in the 6 mm. and 8 mm. embryos but without success. It has not been possible, therefore, to trace these cells back to neural crest origin. The same sort of difficulty has apparently occurred to other students in connection with the whole postganglionic sympathetic system.

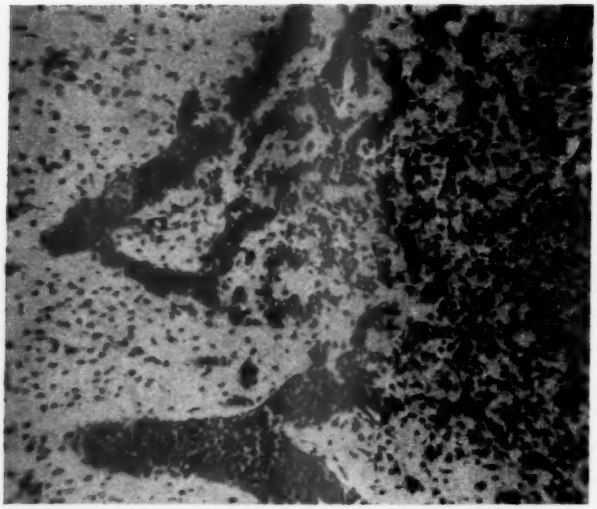

FIG. 3. The vascular plexus of the undifferentiated falx cerebri in sagittal section of a 20 mm. human embryo.

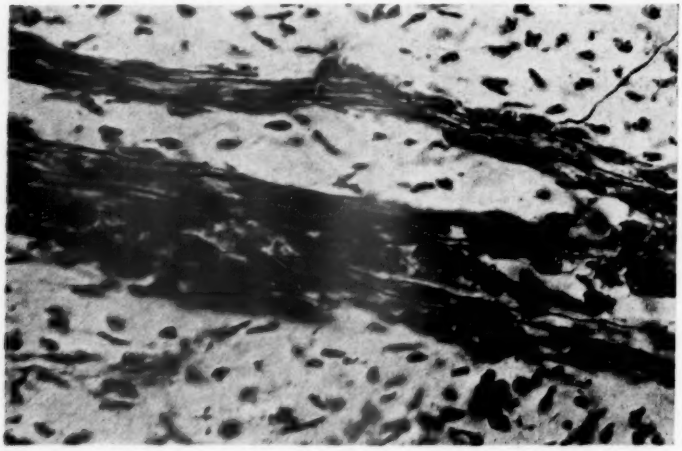

FIG. 4. Detail of the olfactory nerve in silvered section of a 20 mm. embryo. Olfactory fibers are dull brown and terminal nerve fibers among them are fine black lines.

Summary

A plexus of nerves and ganglia found in the falx cerebri of the human infant extends across the midline in places. It is traced back in its development from the period of birth successively to the 43 mm., 20 mm., and 12 mm. stages of the human embryo. It shows evidence of being developed along the course of the ophthalmic branch of the trigeminal nerve. It is from the first identified with a vascular plexus which moves with it into an intracranial position. The earliest stages of the cells provide no clear cytological identification as nerve cells even in silver impregnated sections in which mature cells and fibers are clearly identified.

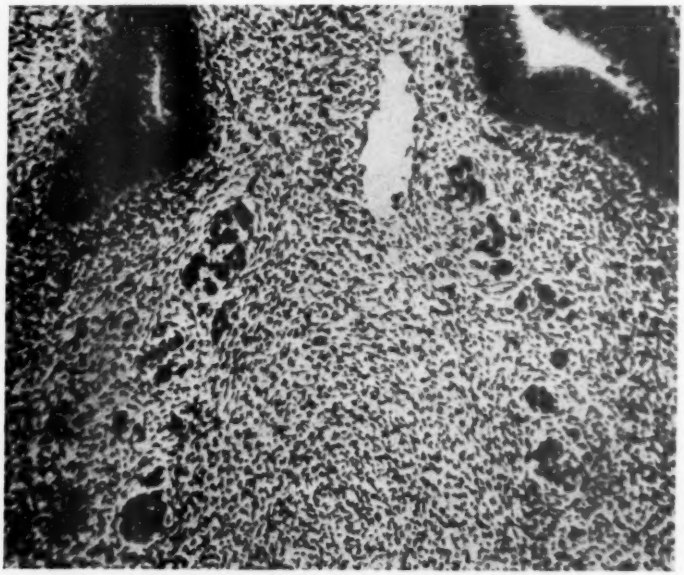

FIG. 5. Cross section of the ethmoid region of an 18 mm. human embryo. Clusters of ganglionic cells are arranged on the dorsolateral borders of the undifferentiated ethmoid between olfactory vesicles.

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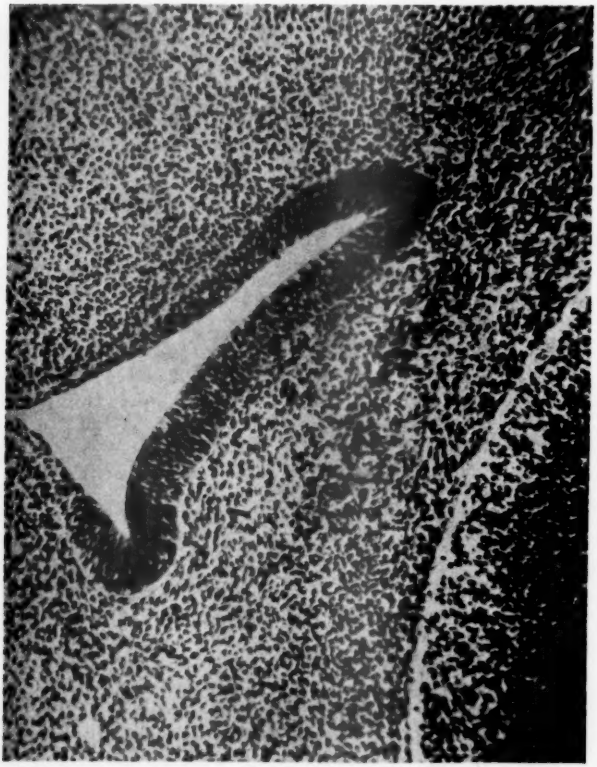

FIG. 6. Primitive nerve cell column lying between the nasal pit and forebrain of a 12 mm. embryo. This is on the course of the ophthalmic fibers of the trigeminal nerve and just dorsal to the transverse olfactory fibers.

TUBULAR HEART AND TRUNCUS ARTERIOSUS IN AN INFANT*

JOHN G. SINCLAIR AND WILLIAM POWELL

Cor biloculare is a term applied to hearts in which both atria and both ventricles are connected because of missing septa. The condition is rare even when the septal defects are small. No case known to us lived into early infancy with the septa nonexistent and the truncus arteriosus showing no signs of division. Wood and Williams report a case, living to 72 hours after birth which approximates our example. It is felt, therefore, that this case represents about the limit for this anomaly compatible with life.

A Mexican infant was hospitalized from the time of birth because of difficulties in feeding. She had a right harelip and a cleft palate of severe degree. She was fed by tube and by the thirteenth day weighed nine pounds having gained two pounds in two weeks. The infant then developed a spiking temperature of 104 degrees F. and began to show muscular twitching. Antibiotic therapy under an oxygen tent did not improve the symptoms and she died on the nineteenth day weighing nine pounds.

At necropsy an external examination disclosed mongoloid facies, cleft palate, and right harelip. There is a large hematoma of the right scalp. The fingernails of the little fingers are missing and the fingers are short. No visceral transpositions are seen. The size and appearance of abdominal and thoracic viscera are normal. The kidneys show marked fetal lobulation. Both ureters and bladder are normal. Ovaries are long and narrow (5 x 32 mm.) and extend cranially on both sides from points 2 cm. above the pelvic brim. The uterus is bicornuate but cervix and vagina are normal.

The heart weighs 57 gms. and is superficially normal except that no pulmonary artery comes from the ventricle. The atrial cavity is single with appendages rather poorly developed.

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*From the Departments of Anatomy and Pathology, The University of Texas Medical Branch. Received for publication July 15, 1951.

The primary septum is represented by a band of tissue attached at the ends as in figure 1. SP.

FIG. 1. Atrium of a nineteen day old human cor biloculare. RA is right atrium. SP is septum primum. V 1-4 are AV valves all to the right of the septal band.

There is no indication of a secondary septum. The four valve cusps are all on the right side of the band each with its papillary muscle extending well down into the common ventricle. The ventricle shows no signs of division. The columna carnae are well developed and form thick supports under each of the three and a fourth diminutive aortic cusp (Fig. 2). From the aortic sinuses above the two left cusps arise the coronary arteries. The surface of the truncus is smooth with no sign of a spiral septum. The continuing left arch is seen in figures 3 and 4. The right arch is a blind sac giving rise to the pulmonary trunk by what was originally the right ductus arteriosus. The only trace of a possible left ductus is a small sac seen in figure 3. The pulmonary trunk just below this point divides into pulmonary arteries. The superior and inferior vena cavae enter the right side of the atrium together with the pulmonary venous trunk.

There was no possibility of even partial separation of oxygenated and deoxygenated blood streams in this heart. It is surprising that the infant lived for nineteen days under such a disadvantage. Had it not been for the palatal defect which resulted in aspiration into the trachea with resulting pneumonia it seemed able to grow and might have lived much longer.

FIG. 2. The ventricle of a nineteen day old human cor biloculare. C shows openings into coronary arteries behind valve cusps in the left half of the common truncus.

This case differs from others which approximate it in the literature in that there is so little transposition or reversal

of symmetry. As is usual in such cases there were other embryonic arrests. No electrocardiographic study was made and the extensive anomaly was not suspected at first examination. Blood pressures were not recorded. In the similar Wood-Williams case the pressures were 80/0. Apparently as long as oxygenated placental blood flows through such a system normal growth continues, but the strain of accommodating to pulmonary ventilation lowers its reserves so that any added strain makes it fail.

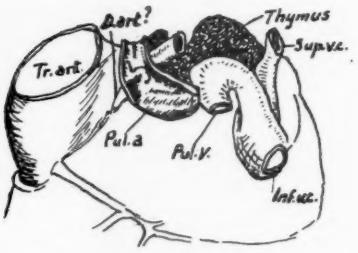

FIG. 3. The dorsal atrial wall of a human cor biloculare.

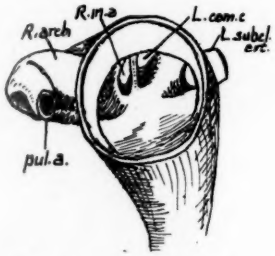

FIG. 4. Truncus arteriosus and branches from a nineteen day old human cor biloculare.

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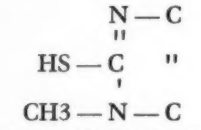
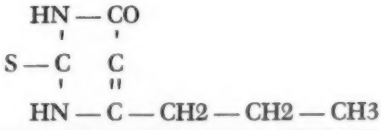
PRE-OPERATIVE USE OF, AND HISTOLOGICAL CHANGES PRODUCED BY TAPAZOLE*

CHARLES T. STONE, JR., JOHN H. CHILDERS, JOHN E. JOHNSON, AND ISADORE V. EPSTEIN

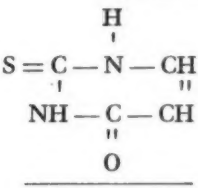
Recent years have witnessed a procession of drugs, all of which are used to reduce over-activity of the thyroid gland. The most recent such drug is "tapazole" (1-methyl 2-mercapto imidazole). It can be seen from the structural formulae below that the fundamental chemical difference between the thiourea compounds and the imidazoles is the presence of a six atom ring in the former instead of a five atom ring as in the latter.



Tapazole



Propylthiouracil



Thiouracil

Mode of Action

The active portion of the thyroid hormone (thyroxin) is synthesized in three stages: conversion of iodide into iodine (oxidation?), iodination of tyrosine to diiodotyrosine, and coupling of two molecules of diiodotyrosine to form thyroxin. Thiouracil inhibits formation of thyroid hormone, by preventing iodination of tyrosine and/or oxidation of iodide to iodine.

*From the Department of Internal Medicine, The University of Texas Medical Branch, Galveston. Received for publication July 20, 1951.

Although the percise mechanism of action is not fully understood, tapazole apparently functions in a capacity similar to thiourea derivatives.

Williams reported that in one series of 119 patients treated with a thiourea derivative, fifty-seven maintained complete remissions ranging from 1.5 to 4.5 years without additional therapy. In general clinical practice, however, at the present time subtotal thyroidectomy is probably the treatment of choice. In this capacity the so-called anti-thyroid drugs may be of invaluable assistance in preparation for surgery.

In the John Sealy Hospital, for at least three years, propylthiouracil has been used as the preparatory drug of choice. Iodine has been used in the immediately pre-operative phase in an attempt to reduce the vascularity of the gland.

Stanley *et al.* compared the anti-thyroid properties of 2-thiouracil, 6-methylthiouracil, 6-n-propythiouracil, 2-mercapto-imidazole and 1-methyl, 2-mercapto imidazole (tapazole). Judged from studies of radioiodine accumulation, tapazole was approximately one hundred times as active as thiouracil weight for weight and only seven times as toxic.

McGavack studied 546 cases of thyrotoxicosis of which 60 were treated with tapazole. It was his opinion that of the several anti-thyroid drugs available, tapazole was generally the most useful. No instances of toxicity were noted.

Although seven cases of thyrotoxicosis have been treated with tapazole, only five lend themselves to adequate study. Of these cases one is of particular interest (2224-G) because, even with modern methods of therapy at hand, save for radioactive iodine, the patient failed to respond to as much as 800 mgms. of propylthiouracil and later 60 mgs. of tapazole per 24 hours, and finally expired under circumstances suggesting hyperthyroid exhaustion.

All patients were at the height of their disease when tapazole was started.

Dosage varied from 10 to 60 mgs. per 24 hours. Instead of the usual three times a day routine, the medicine was administered at 6 or 8 hour intervals. No instances of proven toxicity were encountered. This report centers around five cases, abstracts of which are included below.

No. 2224-G: B. L., a 53-year-old Negro female, was first seen February 1, 1950, complaining of swelling in the neck, palpitations, pain in the left side, weight loss in spite of a good appetite, tremor and intolerance to heat. These symptoms had begun about one year previously and she had been treated with "white tablets" which made her nauseated. She was placed on Lugol's solution, drops six daily, in July, 1949. A diffuse goiter was present with bruit. The BP was 162/100 and P 142. She was admitted to the hospital on March 15, 1950, where it was noted that she had developed auricular fibrillation. Her pulse was 110 to 145, BP 150/80 and BMR was plus 44. Exopthalmos was present. Blood count revealed 10.2 gm. Hb., 4.09 million RBC's, 7,200 WBC's with 59 PMN's, 1 stab, 32 lymphs, and 8 monocytes. She was started on 100 mg. of propylthiouracil three times a day (9, 1, 5) and digitalis leaf. The heart rhythm became regular on April first, but remained rapid. The BMR remained high, the last reading being plus 52 before she was discharged on April 19, 1950, to be followed in the outpatient department. During this hospitalization, the WBC fluctuated between 3,100 and 5,500; the PMN'S decreasing to 42, then 27, and remaining low, regardless of the total white blood count.

The patient was seen at intervals in the clinic and seemed to be subjectively improving except for one period of six weeks during which medication was omitted. She was never thought quite ready for surgery, however, and on August 21, the BMR was plus 53.

She was readmitted to the hospital on September 20th and placed on Lugol's solution with continuation of propylthiouracil 250 mg. daily in divided doses, usually given at 9, 1, 5, 9. She continued to gain weight, but the pulse rate remained above 100 during the day; pulse was 90–100 during sleep; and the BMR remained above plus 40. Propylthiouracil was increased to 400, 600 (Lugol's discontinued at this time) and 800 mg. per day without effect on pulse rate and BMR. On November 8, 1950, tapazole 10 mg. every eight hours was begun. At this time the BMR was plus 50. On November 9, tapazole was increased to 10 mg. every six hours and on November 17, to 15 mg. every six hours. On December 2, 1950,

total WBC's were 2,800 with 42 PMN's, 1 stab, 53 lymphs, 2 monocytes, and 1 eosinophil. Previous counts had ranged between 4,300 and 6,250 during this admission with a similar or lower proportion of PMN's. Tapazole was discontinued but resumed on December 4, 1950, when WBC's of 5,350 were obtained. On December 7, 1950, the patient developed dyspnea with signs of pulmonary edema, a very rapid heart rate, shown to be auricular flutter with 2:1 block, and increasing disorientation. All therapeutic measures failed including oxygen, sedation and rapid digitalization, and the patient expired under conditions suggesting hyperthyroid exhaustion.

Autopsy Findings: P. M. 8344. B. L. was a 54-year-old colored female, measuring 5 feet and 7 inches (167.5 cm.) and weighing an estimated 130 pounds (59 kilos) when examined eight hours after death. The body had not been embalmed and rigor mortis involved all four extremities. No prominence of the eyes was noted. There was an internal strabismus of the right eye. A diffuse enlargement of the thyroid gland was noted but no other masses were palpated in the neck.

The pleural cavities were devoid of free fluid. Firm, fibrous adhesions lay between the visceral and parietal pleurae of the apical and basalar portions of the right lung. 15 ml. of clear, straw-colored fluid were present in the pericardial cavity.

The heart weighed 360 grams. Several petechiae were noted in the epicardium and ecchymoses were present along the ascending aorta as well as along the posterior wall of the left atrium. The coronary arteries were slightly sclerotic but retained a smooth intimal surface. The flabby, soft myocardium measured 18 mm. in thickness in the left ventricle and 3 mm. in thickness in the right ventricle. No endocardial gross changes were noted. Microscopic studies of the myocardium showed parenchymatous degeneration of the cardiac muscle. Patchy areas of increased fibrous connective tissue were scattered throughout the wall.

The right lung weighed 410 grams and the left lung weighed 560 grams. A serosanguinous fluid dripped from the cut surfaces. The parenchyma was soft and had a reddish-purple

color. The most impressive histological changes in the lungs were the marked congestion of vessels, even in the alveolar wall. In one area, the alveolar spaces contained red blood cells as well as pink staining fluid.

The diffusely enlarged thyroid gland weighed 120 grams. The lobular surface was covered with a well-defined, thin, transparent capsular membrane. Numerous thin-walled vessels coursed through this tissue. Upon sectioning, the thyroid had a beefy-red, firm-cut, slick surface. A few strands of grayish-white tissue extended between the lobules. No areas of hemorrhage or necrosis were encountered. Near the lower poles of the right lateral lobe was an oval, discrete, white nodule 4 mm. in diameter. This nodule was incorporated within the thyroid and was 1.5 cm. from the margin.

The three parathyroid glands found averaged 3 mm. in diameter. Microscopic examination of several sections from the thyroid showed a marked variation in the size of the acini. A few of these spaces were filled with a variable staining homogenous or vacuolated colloid, while many acini contained deeply acidophilic staining homogenous colloid. The lining epithelium varied from tall columnar to cuboidal, and numerous papillary folds, covered with columnar epithelium, projected into the luminae of the acini. The small acini contained a faintly acidophilic staining, vacuolated colloid. A few focal collections of lymphocytes and large mononuclear cells were present in the interstitial tissue. In some of these areas recognizable germinal follicles were present. The vessels were markedly engorged with erythrocytes (Fig. 1). The colloid stained green with the Masson's trichrome stain. The circumscribed, 4 mm. nodule in the right lobe was composed of cords of cells that assumed the histological pattern of a microfollicular adenoma.

The abdominal viscera were in their usual anatomical location. The liver weighed 1,610 grams. The capsular surfaces were smooth and glistening. Cut surfaces were reddish-gray in color and sectioned with usual resistance. Ten ml. of dark amber, mucoid material was found in the gallbladder. Near the center of the hepatic lobules the cord cells contained small

vacuoles. These intracytoplasmic spaces were stained for lipoid with Sudan IV strain.

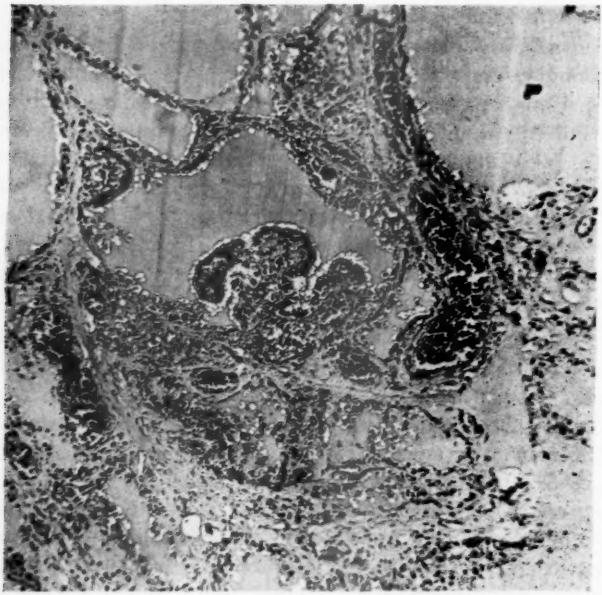


FIG. 1. Hyperplastic thyroid treated intensely with propylthiouracil and subsequently with tapazole. Papillary overgrowth of columnar epithelium was prominent. X 100.

The weight of the spleen was 70 grams. The smooth slate-gray color was not remarkable. Upon sectioning, the soft red pulp scraped away with ease. Several petechial hemorrhages were noted on the mucosal surface of the stomach and proximal portion of the duodenum. This hemorrhage was limited to the superficial portion of the mucosa; although, all of the vessels were congested. The lobular pancreas had a grayish-pink color and was sectioned with ease. Examination of the adrenal glands, atrophic uterus, fallopian tubes, ovaries, cervix, vagina, skeletal muscles, lymphatics, and skeletal system showed no pertinent gross changes. The right and left kidneys weighed 160 grams each. The pelvis, ureters and urinary bladder were

not grossly remarkable. The tubular epithelium showed a vacuolization of the cytoplasm. These vacuoles were stained for lipoid with the Sudan IV stain.

The brain weighed 1,360 grams. The gyri were flattened but numerous sections showed no additional gross changes. Sections from the cerebral cortex, basal ganglia, pons, medulla oblongata, and cervical spinal cord, showed no pertinent histological changes when studied with routine hematoxylin and phloxine stained sections.

No. 5930-G: F. G., a 49-year-old Latin-American female, was admitted to the hospital on August 15, 1950, complaining of headache, nervousness, sweating, shakiness, heat intolerance, and sleeplessness. The precise onset of these complaints was apparently at, or subsequent to May, 1950. Examination showed a well-nourished, middle-aged female with moist skin, tremor of the hands, a diffusely enlarged thyroid gland, and BP of 240/120. Cholesterol was 186 mg% and BMR was plus 53. She was started on propylthiouracil 300 mg. per day. The BMR fell to plus 22. She was placed on Lugol solution along with propylthiouracil, and she was dismissed from the medical service on September 10, 1950, to be called in for surgery as soon as a bed was available. She was seen six weeks later at which time considerable toxicity had returned clinically, although the BMR had risen only to plus 30. Lugol's solution was discontinued.

In early November she became negativistic, mute and agitated and had to be admitted to the Galveston State Psychopathic Hospital where propylthiouracil was discontinued. She subsequently received a course of electroconvulsive therapy. Her normal personality had returned by the latter part of December; and on the advice of the medical consultants, she was started on Lugol's solution and shortly afterward, she was transferred to the surgical service. Because her BMR was found to be plus 47, she was sent to the medical service for further preparation. On January 20th tapazole was begun 5 mg. every six hours. The BMR at first remained essentially unchanged; and on February 7th, dosage was increased to 10 mg. every six hours. Within a week the BMR had fallen to plus 23; subsequent readings being plus 26, plus 17, and

plus 20. The cholesterol levels were probably not significant consecutively 170, 206, 180, 260, 210 mgm%). This patient was ready for operation by the first week in March, although it was not done until March 23. At one time this patient complained for several days of a persistent numbness and tingling of her hands, which was thought to be related to some handwork she had been doing rather than to medication.

FIG. 2. Preoperative preparation consisted of tapazole. Acinar structures show considerable variation in size were lined with a low columnar to cubiodal epithelium. X 100.

Operative Findings: A subtotal thyroidectomy was performed on March 23, 1951. The gland was firm and rubbery. It did

not appear particularly vascular grossly, although bleeding was somewhat troublesome during surgery and for a short time afterward. The pulse rose to 160 and the blood pressure to 250/160 millimeters of mercury. Since respiratory obstruction had been present to a varying degree during most of the procedure, the increased pulse and blood pressure with the increased tendency to bleed was probably not the result of the antithyroid drug used.

Surgical Pathology: S-29457. The specimen from the right and left lobe of the thyroid measured 5.5 by 3.5 by 1.2 cm. and 5 by 3.8 by 1.5 cm. and had a combined weight of 25 grams. The dorsal, cut surface was shaggy. The convex surface was lobular and covered by a pliable, thin, transparent membrane. Many blood vessels coursed through this soft tissue. The tissue had beefy-red, glistening, sticky cut surfaces. No areas of degeneration were encountered in the specimen. The microscopic sections showed the larger acini to be filled with a deeply acidophilic staining colloid while the smaller acini contained sheets of inter-acinous cells (Fig. 2). Diffuse and focal infiltrations of the interstitial tissue by lymphocytes, large mononuclear and a few plasma cells were noted. Some of the aggregates of lymphocytes contained large, oval, germinal centers. The colloid stained either red or green with the Masson's trichrome stain but most of the acinar spaces contained green staining colloid.

No. 14761-G: M. B., a 31-year-old Latin-American female, was admitted to the hospital on December 16, 1950. In December, 1949, she noted a "ball" in her stomach which caused nausea and vomiting. This later subsided with the development of nervousness and weight loss. Questioning elicited the story of diarrhea and heat intolerance; but due to language barriers, it was impossible to obtain further pertinent history. Examination revealed a diffusely enlarged thyroid gland, P 100 and BP 120/50. The patient was somewhat apprehensive and tremulous and slight exopthalmos was present.

BMR was plus 50, serum cholesterol 150 mg. per 100 ml., CBC: Hb. 12.3 gm., RBC's 5.42; WBC's 6,650; PMN's 66; lymphs 23; monocytes 8; eosinophiles 3. Tapazole 10 gm. four times per day (9, 1, 5, 9) was ordered for December 20. The

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dose was selected not because of the severity of the disease, but in an effort to reduce the time required for therapy. On eight of the succeeding 21 days, the patient failed to get the full dose. On January 9, 1951, the dose was increased to 15 mg. every six hours. The BMR fell steadily, and on January 15, was plus 15. The white count was unchanged; the cholesterol rose to 266 mg.% on January 8 and the patient gained 17 pounds. Her pulse rate had always ranged between 80 and 110 and was unchanged; but she was subjectively less nervous and her tremor had disappeared. She was submitted to surgery on January 17, 1951.

Operative Findings: A subtotal thyroidectomy was performed on January 17, 1951. The gland was diffusedly enlarged, soft and quite vascular.

Comment: The post-operative course was uneventful and the patient was discharged on the fifth post-operative day. This case would suggest that tapazole alone is inadequate to control the increased vascularity if given for only a short period (four weeks). It was adequate to control the altered physiology of metabolism, however. The large dosage of 60 milligrams daily was used to test the tolerance of the patient. There were no untoward reactions to this dosage.

Surgical Pathology: S-28619. The right portion of the "U-shaped" mass of thyroid tissue measured 5.5 by 3.5 by 2.5 cm. and the left portion measured 5.5 by 2.5 by 2 cm. The total weight was 45 grams. Numerous variable sized nodules were present on the smooth convex surface. This reddish-gray, beefy, cut surface was divided by septae of grayish-pink tissue. Histologically most of the small acini contained a small amount of vacuolated, variable staining colloid. The lining epithelium was low columnar epithelium (Fig. 3). Small clusters of lymphocytes were present in the delicate, narrow strands of connective tissue of the stroma. The colloid stained green with the Masson's trichrome stain.

No. 13132-G: E. R., a 27-year-old white female was first seen in the clinic on October 16, 1950. Her history of nervousness, irritability, weight loss, heat intolerance, sweating, hot flashes, headaches, palpitations, and difficulty in swallowing

had begun about September, 1949. In September, 1950, she was given Lugol's solution for two weeks, during the second week of which, a skin rash appeared on the extensor surfaces of the arms and legs. This disappeared when iodine therapy was discontinued. Examination revealed a rather apprehensive tremulous young, white female with rather pronounced exopthalmos, moist skin, P 128 and BP 110/70. BMR was plus 40, urinalysis was normal, CBC: Hb. 11.6 gm.; RBC's

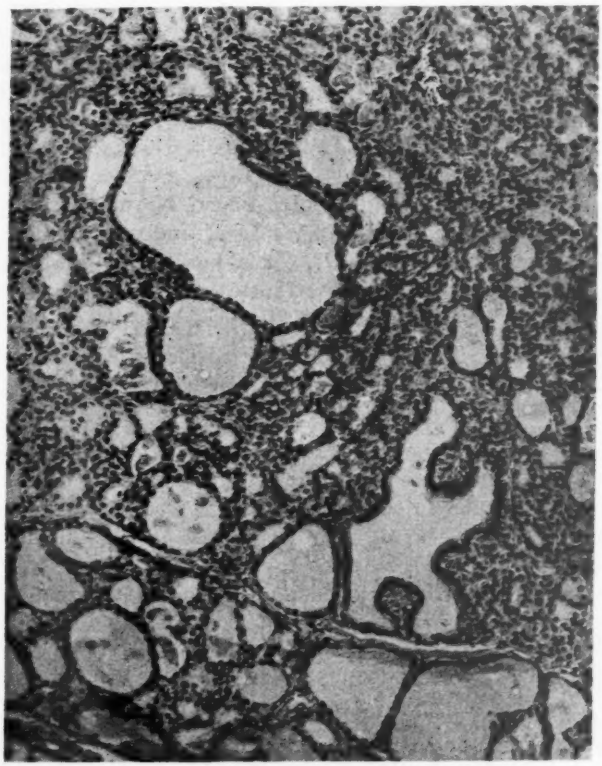

FIG. 3. **Hyperplastic goiter that was treated with tapazole. The small acini contained no colloid or a small amount of vacuolated, variable staining colloid. X 100.**

4.56M; WBC's 5,600; PMN's 47; stabs 3; lymphs 47; monocytes 1, and eosinophiles 2. Cholesterol was 143 mg. per 100 ml.

Tapazole, 5 mg. every 12 hours, was begun on October 25 simultaneously with phenobarbital. Two days later she developed a maculopapular pruritic rash on the neck, upper extremities and sacrel region. There had been a few vesicles at the onset. The rash cleared over a period of several days after phenobarbital, but not tapazole, was discontinued. The BMR fell to plus 32, then rose to plus 42 (possibly in association with emotional stress of caring for a sick friend). An upper respiratory infection followed shortly thereafter, which was treated successfully with penicillin. On November 14, the patient stated she felt much improved and less nervous. The pulse rate was 84 and almost daily checks during the following week revealed the pulse always less than 90, so that despite the BMR reading of plus 23 on November 18, the patient was considered ready for surgery and was admitted to the hospital on November 21. Cholesterol readings were not significantly changed nor were urinalysis or blood counts. The post-operative BMR was 5%.

Operative Findings: A subtotal thyroidectomy was performed on November 21, 1950. Bleeding was decreased from that usually seen in a routine thyroidectomy.

Comment: The post-operative course was uneventful and the patient was discharged on the fifth post-operative day. The decreased vascularity seen in this patient was an encouraging finding. It was difficult to attribute this fact to the iodine administered five weeks prior to surgery. This patient was adequately prepared by the administration of tapazole for a four-week period. This case also is interesting in that the patient was allergic to iodine and phenobarbital, but was able to take tapazole without undesirable reaction.

Surgical Pathology: S-28088. The thyroid tissue removed from the right and left lobes measured 5.5 by 4 by 2.5 and 5.5 by 3 by 2.2 cm. The combined weight was 47 grams. A convex surface of each mass was covered with a transparent, grayish, thin membrane. Numerous blood vessels coursed over

the surface of the reddish-brown, nodular tissue. The cut surfaces were divided into lobules by narrow grayish-white septae. Microscopic sections from various areas showed essentially the same histological changes. The acini were lined with low columnar to cuboidal epithelium and contained a few vacuoles of the pink staining colloid material when stained with hematoxylin and phloxine (Fig. 4). Some of the acini showed delicate papillary projections into the lumen, with these projections being covered with columnar epithelium. The small acini were lined with a columnar epithelium. This colloid stained green with the Masson's trichrome stain. A few collections of lymphocytes and mononuclear cells were present in the interstitial tissue.

No. 4285-D: J. V., a 46-year-old male was first seen on May 9, 1950, complaining of nervousness, weakness, weight loss in spite of good appetite, goiter, glassy eyes, exertional syncope, sweating, and heat intolerance. The onset was probably about two years previously. Examination revealed a nervous white male perspiring freely, with fine tremor of the hands, exophthalmos, diffuse enlargement of the thyroid gland. The pulse was 80 (regular) and the BP 140/75. Initial blood count revealed Hb. 13.2 gms., RBC's 4.2 million, WBC's 6,350 with PMN's 64, stabs 2, lymphs 30, monocytes 3, eosinophiles 1. Cholesterol determinations were 180 and 234 mg. per 100 ml., one week apart.

BMR on June 1st was plus 30. The patient was placed on 50 mg. propylthiouracil four times a day and followed as an outpatient without bed rest. BMR's remained unchanged and the patient was unable to work because of his symptoms. On December 20 his BMR was plus 53 and propylthiouracil was increased to 500 mg. per day for seven days; then decreased to 40 mg. per day. He was admitted to the hospital on January 12, 1951, and tapazole, 5 mg. every six hours was started January 17, at which time his BMR was plus 32. On January 25 his BMR was plus 19 and he felt subjectively better; however, on February 7, his BMR was plus 44 and he seemed to be losing ground. Tapazole was increased to 10 mg. five times a day. The pulse rate varied between 80 to 110. The BMR rose to plus 50, then gradually fell to plus 23 on March 9,

1951. The pulse was usually below 90 in the two weeks prior to surgery. Lugol's solution was begun on February 28th. He failed to gain weight and was not entirely symptom free at the time of surgery. There was no significant change in the white blood count or urinalysis (always normal) and blood cholesterol levels in the hospital were 250, 266, and 224 mg.%.

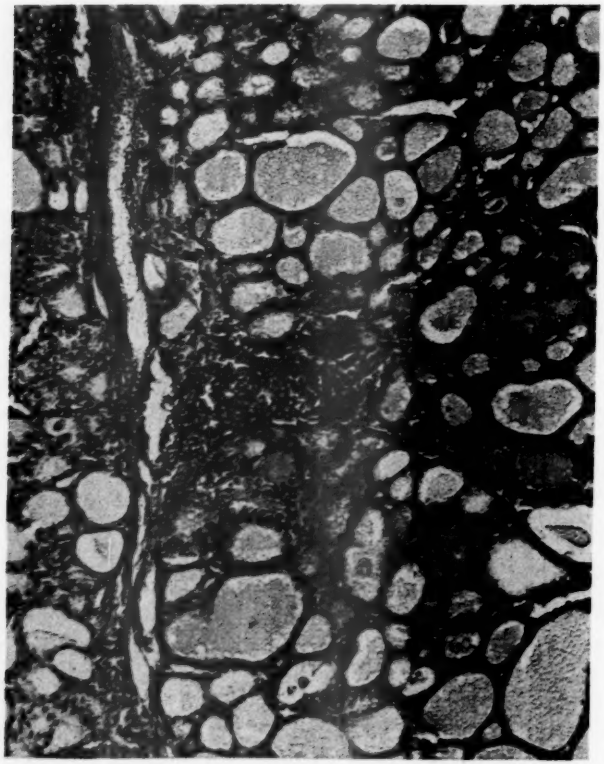

FIG. 4. Note the aggregrates of lymphocytes and irregular staining colloid in a diffuse hyperplasia of thyroid gland treated with tapazole. X 100.

Operative Findings: A subtotal thyroidectomy was performed on March 21, 1951. The thyroid gland was soft, friable, and

extremely vascular. Bleeding was comparable to that seen with the combined preparation with iodine and propylthiouracil.

Surgical Pathology: S-29428. The specimen obtained from the left lobe of the thyroid weighed 72 grams and measured 8.5 by 6 by 2.8 cm. Numerous dilated vascular channels coursed over the surface. Upon sectioning, a firm, uniform reddish-gray, slick cut surface was noted. Tissue from the right lobe of the thyroid measured 9 by 6 by 2 cm. and weighed 56 grams. The cut surfaces were similar in appearance to the tissue from the left lobe. A firm, rectangular mass of tissue from the isthmus of the thyroid weighed 12 grams. The total weight of excised thyroid gland was 140 grams. The microscopic sections showed considerable variation in the size and shape of the acini (Fig. 5). The infolds of epithelium were columnar while the remaining portion of the acinar wall was formed by cuboidal epithelium. A pink staining colloid filled the lumen of the acinar spaces. The colloid had smooth margins but contained a few vacuoles. An occasional small clump of lymphocytes and large mononuclear cells was present in the narrow strands of connective tissue stroma. The colloid stained uniformly green with the Masson's trichrome stain.

Comments

This series of cases is too small to permit generalizations, but it is felt that there are several interesting features brought out in the study of these patients. Although the dosage varied widely, it was possible to prepare one patient for surgery on a dosage of 20 milligrams per day for a period of one month. The maximum dosage given to M. B. was 60 milligrams per day for eight days, and to B. L. the dosage was the same for twenty-three days. There was no evidence of toxicity observed. However, with one patient (B. L.), the white blood cell count fell to 2,800 on one occasion. This count was checked forty-eight hours later and found to be 5,350 which was essentially the same with no granulocytopenia being observed before or during the leukopenia.

A study of the post mortem specimens in the case of B. L. (2224-G) showed hyperplasia of the thyroid gland, hypertrophy of the heart when calculated on the basis of the patient's

weight prior to illness, minimal degenerative changes in the liver and fatty degeneration of the renal tubular epithelium. Previous autopsy studies of patients dying of hyperthyroid crisis have revealed no precise organic changes to account for the patient's death.

FIG. 5. Hyperplasia of thyroid gland prepared with tapazole and iodine. The colloid stained deeply acidophilic with the hematoxylin and phloxine stain but vacuoles in colloid and a few papillary folds of columnar epithelium were noted. X 100.

The significance of the petechial hemorrhage of the mucosa of the stomach and adventitia of the thoracic aorta could not be definitely stated. These were accompanied by tremendous

congestion of all the viscera. Histologic studies of these areas indicated that they were recent or terminal events. These may have resulted from anoxia or perhaps from the drug (tapazole?). The hyperplastic changes in the thyroid gland persisted although she received intensive therapy.

The lack of evidence of toxicity in the patients treated to date with tapazole would indicate that long term control of these patients can more accurately be accomplished with this drug than with any other anti-thyroid preparation.

Bleeding observed at the time of surgery has varied in this small series of four cases. In one instance (E. R.) tapazole alone was sufficient to relieve the toxicity and reduce the vascularity of the thyroid. In a second patient (F. G.) there was decreased vascularity. However, this patient had received propylthiouracil and iodine prior to the institution of tapazole therapy. In the two remaining patients that were operated upon, the vascularity was a definite hazard predisposing to excessive bleeding.

Sections from each case were stained with hematoxylin and phloxine, Masson's trichrome and Heidenhains' azocarmine. In the thyroid glands of patients prepared with tapazole, some of the colloid stained irregularly, was vacuolated, and presented scalloped edges. In other areas the large acini contained deeply acidophilic staining non-vacuolated colloid in the hematoxylin and phloxine stained sections. Hyperplastic epithelium lined the acini and formed papillary projections. These changes were similar to the change seen in hyperplasia of the thyroid with involution.

In the thyroid glands treated with tapazole and iodine the involution was more marked with the colloid assuming a homogeneous, deeper acidophilic staining. Most of the acini were lined with a low columnar to cuboidal epithelium.

These observations indicated that tapazole acted to inhibit the formation of normal colloid in the cases studied. When iodine and tapazole were combined, the faulty maturation was corrected, the colloid assumed mature staining characteristics, and the involution was more marked.

Summary

Tapazole is an effective anti-thyroid drug. No proven instances of toxicity have been observed to date. One death from hyperthyroid exhaustion is presented and the post mortem findings have been discussed. Subtotal thyroidectomy was performed in the remaining four patients. Histological changes in the surgical specimen removed at the time of operation have been described.

Addendum

Since the original material was compiled, fifteen additional cases of Graves Disease have been treated with tapazole. Six patients were operated upon without iodine preparation. Bleeding was excessive in all six instances. Two patients developed a maculopapular rash. In both cases tapazole was continued, in reduced dosage, and in seven to ten days, the rash had completely disappeared. One patient developed a profound granulocytopenia, which spontaneously corrected itself following cessation of tapazole. The largest dose in any one 24-hour period was 80 milligrams. These additional fifteen cases make obvious the fact that tapazole is a remarkably effective antithyroid agent, although its use must be closely supervised.

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EFFECTS OF STRESSES ASSOCIATED WITH FLYING ON THE CARDIOVASCULAR-RENAL LESIONS PRODUCED BY HYPERSENSITIVE REACTIONS*

I. Effects of Acute Altitude Exposure on the Cardiovascular-Renal Lesions in Hypersensitive Animals

Ralph M. Thompson, Anna C. Arnold, and Roland B. Mitchell

Introduction

The relationship of allergic (hypersensitive) factors to the pathogenesis of the collagen diseases has been the subject of several recent reviews (Duff, 1948; Waksman, 1949; Lapin and Starkey, 1949; Klemperer, 1950). The detrimental effects of exposure to altitude on animals sensitized to a foreign protein were demonstrated by Lumière and Couturier (1923) and by Kopaczewski and Marczewski (1935). These workers found in sensitized animals an increased susceptibility to the effects of decompression. The pathologic picture incident to hypoxia has been well established. The first graphic description was made by Glaisher (1862) following his balloon ascensions with Coxwell. The excellent papers by Kritzler (1944) and by Lewis and Haymaker (1948) covered the subject in detail. Exposure of experimental animals to hypoxia at simulated altitudes in a decompression chamber has resulted in such changes as cardiac hypertrophy, fatty metamorphosis, infarction, fibrosis, and other histologic evidence of severe myocardial damage. Thickening and inflammation of the atrioventricular valves were reported by Dalton *et al.* (1945) and later confirmed by Highman and Altland (1949).

Many reports have appeared concerning the resemblance between the lesions of periarteritis nodosa, rheumatic fever, and rheumatoid arthritis in humans, and those produced in experimental animals sensitized by parenteral injections of foreign proteins (Rich and Gregory, 1943; Rich and Gregory,

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1943; Rich and Gregory, 1944; Hopps and Wissler, 1946; Smith and Zeek, 1947; Moore *et al.*, 1947; Hawn and Janeway, 1947; McKeown, 1947; Zeek, Smith, and Weeter, 1948; Ehrich, Seifter, and Forman, 1949).

Clark and Kaplan (1937) emphasized the similarity of the pathologic lesions of serum sickness in man with those of the rheumatic diseases. Rich and Gregory (1943; 1943; 1944) reported that periarteritis nodosa can result from protracted hypersensitive reactions of the anaphylactic type. This vascular lesion can be produced experimentally by subjecting animals to anaphylactic reactions of the "serum sickness" type, and in its advanced and fibrous form may become indistinguishable from ordinary arteriosclerosis.

This report gives results of an exploratory experiment which attempted to point the way to further research on the effects of exposure to altitude on the cardiovascular-renal lesions produced by protracted hypersensitivity. Attention is focused primarily on the effects of intermittent exposure to acute hypoxia produced at simulated high altitudes on the pathologic changes resulting from protracted hypersensitive reactions.

Experimental Procedure

Male albino rabbits ranging in weight from 1,825 to 2,900 grams were employed in this study. Four treatment groups of 15 animals each were used with three replications of 5 rabbits for each of the treatment combinations as follows: group 1, comprising the normal controls; group *a*, receiving serum only; group *b*, receiving stress only; and group *ab*, receiving both horse serum and intermittent stress associated with decompression.

To sensitize each of the rabbits in groups *a* and *ab*, 10.0 ml. of sterile horse serum per kilogram of body weight was given as a single injection into an ear vein according to the method of Rich and Gregory (1943).

All animals in groups *b* and *ab* were subjected to decompression in an altitude chamber for a 2-hour period each day from Monday through Friday for 2 weeks. This exposure to altitude was started on the 13th day after horse serum was

administered to animals in groups *a* and *ab*. The simulated altitudes and lengths of exposure for groups *b* and *ab* are given in Table I.

TABLE I

Duration of Exposures to Varying Simulated Altitudes for Animals in Experimental Groups a and ab

Day of exposure	Simulated altitudes (in thousands of feet) Exposure time in minutes											
	12,000	14,000	16,000	18,000	20,000	21,000	22,000	23,000	24,000	25,000	26,000	28,000
1–12	0	0	0	0	0	0	0	0	0	0	0	0
13	30	..	90	..	..	..	..	..	..	..	..	..
14	..	..	30	90	..	..	..	..	..	..	..	..
15	..	..	..	15	15	..	90	..	..	..	..	..
16	..	..	..	..	15	..	15	..	90	..	..	..
17–18	0	0	0	0	0	0	0	0	0	0	0	0
19	..	..	..	10	10	..	10	..	90	..	..	..
20	30	..	..	..	..	..	..	..	..	..	90	..
21	..	..	..	5	5	..	..	10	..	..	90	..
22	..	..	..	5	10	..	..	10	..	..	60	27
23	..	..	..	..	..	..	..	..	..	..	120	..
24–25	0	0	0	0	0	0	0	0	0	0	0	0
26	..	..	..	..	46	..	..	..	..	..	39	..
27	..	..	..	..	..	..	..	..	..	..	90	..
28	0	0	0	0	0	0	0	0	0	0	0	0

Each rabbit in the experimental groups was weighed at the beginning of the experiment and again at 7, 14, 21, and 28 days. On the 12th day each of the 30 animals in groups *a* and *ab*, 6 animals in the control group 1, and 5 animals in group *b* received an intracutaneous injection of 0.1 ml. of horse serum. Observations were made at the end of 24 hours for the type and degree of skin reaction. On the day before the animals were sacrificed, whole blood specimens were obtained from ear veins to provide serums for precipitin titer determinations.

This study was continued for 4 weeks. Animals in groups 1 and *a* were sacrificed on the 27th day; those of groups *b* and *ab*, on the 28th day.

On completion of the experiment all surviving animals were sacrificed by decapitation with guillotine and autopsied immediately. Sections of all organs were immediately immersed in buffered 10 percent formalin for fixation purposes.

Thirteen animals, which received neither stress nor foreign protein injections, served as controls.

In this study emphasis was placed on the pathologic lesions of the heart and kidneys. One of the primary interests was a qualitative estimation of the degree of periarteritis nodosa with associated acute glomerulonephritis and various pathologic criteria indicative of rheumatic carditis. In groups *b* and *ab*, both of which received the added stress of high altitude, the liver and brain tissues were also studied to further evaluate and arrive at a qualitative estimation of the degree of pathologic change incident to acute altitude stress. The animals comprising groups 1, *a*, *b*, and *ab* were studied separately and the degree of pathologic severity tabulated, particularly that of the heart and kidneys, to ascertain the degree of damage incident to altitude stress, hypersensitivity, or a combination of both.

On the basis of these studies, a qualitative estimation of the degree of pathologic severity of the lesions resembling periarteritis nodosa, rheumatic carditis, acute glomerulonephritis, and hypoxia was made and tabulated as 1 to 5 plus. The 60 animals were divided into four main groups as previously stated: group 1, comprising the normal controls; group *a*, receiving serum only; group *b*, receiving stress only; and group *ab*, receiving both horse serum and intermittent stress associated with decompression.

In addition to the gross observations at autopsy, examination was made of histological sections stained with hematoxylin and eosin and Mallory's phosphomolybdic acid hematoxylin.

The following pathologic criteria formed the basis for the diagnosis of periarteritis nodosa. The various pathologic lesions consisted of edema of the arterial media with separation and spreading of the muscle fibers; hyperplasia of the intima layer; fibrinoid swelling and alteration of the intima and media with further splitting of the fibers; necrosis and hyalinization of media and adventitia; perivascular infiltration with polymorphonuclear leukocytes and monocytes, as well as endothelial proliferation; and general vascular sclerosis with ultimate occlusion.

The diagnosis of rheumatic carditis was based on observation of the following: adventitial histiocytes and connective tissue cells with typical Aschoff cells in nodular formation,

along with swelling and fusion of the collagenous fibers or fibrinoid degeneration; the presence of nodular thickening of the atrioventricular valves with cellular infiltration; subintimal and medial deposition of fibrinoid material with infiltration of basophilic granules; mononuclear and, less frequently, bizarre giant cells. Present, but to a lesser extent, were focal lacerations of the cardiac muscle and edema with swelling and fiber degeneration to the point of actual exudation.

In those cases where a diagnosis of acute glomerulonephritis was made, the following were noted in varying degree: pathologic lesions, such as cellular condensation in the glomerular tufts; syncytial proliferation of the glomerular epithelium; hemorrhage in both the Bowman's capsule and the tubular lumina; albumin and casts in the tubules; colloid droplets in the tubular epithelium; hyalinization of the glomerular loops; and exudation.

The pathologic picture incident to hypoxia has been well established as severe cardiac damage secondary to cardiac hypertrophy with right ventricular dilatation, fibrous tissue proliferation, fatty metamorphosis, infarction, and irregular and nodular thickening of the atrioventricular valve leaflets or valvulitis. Congestion, capillary engorgement, scattered subepicardial petechiae, and focal interstitial or larger parenchymal hemorrhages in the heart and kidneys have also been attributed to hypoxic states. One of the pathologic findings considered pathognomonic of hypoxia is the presence of rounded, oval, or slightly irregular vacuoles ranging from 2 to 14 microns in diameter. Their number per cell varies. While many are clear, some have a pale, diffuse pink eosinophilic appearance. Some have a granular appearance, and others contain small and bizarre inclusion bodies. These vacuoles are predominant in the liver, and appear to a lesser extent in the myocardial fibers and in the kidney parenchyma. Even though Pichotka (1942) first described these vacuoles, their exact nature and meaning have not yet been firmly established.

Results

Tables 2 and 4 show that the control animals and those exposed to altitude alone did not develop hypersensitivity to

horse serum within 2 weeks after the experiment was started. All of the rabbits receiving intravenous injections of horse serum developed hypersensitivity as shown by the positive skin reactions listed in Tables III and V.

The results of the precipitin tests for circulating antibodies are presented for each of the experimental groups in Tables II to V. All rabbits receiving intravenous injections of horse serum showed high anti-horse-serum titers. At the end of the study, 8 of the 12 animals given 0.1 ml. skin tests, in the control group 1 and in the group exposed to altitude only, *b* showed the presence of circulating antibodies for horse serum.

The weights of the rabbits in the four experimental groups at the beginning of the study and then at 7, 14, 21, and 28 days are given in Tables II to V.

According to the pathologic findings, the severity of damage in the four groups of animals was estimated qualitatively and diagnosis made under the clinical headings of periarteritis nodosa, rheumatic carditis, acute glomerulonephritis, and hypoxia (Tables II, III, IV, and V).

A quantitative estimate of the pathologic injury that occurred in each of the four groups of experimental animals is given in Table VI.

Various stages of pathologic process were encountered in the sections depending on their group category and severity. Definite and varied histopathologic findings were necessary for positive diagnosis.

The animals comprising the control group (Table II) received neither stress nor serum. Animals 57 and 53 died before the completion of the experiment, both as a result of an intense gastrointestinal infection, and were removed from the statistical study. Autopsy on the 13 animals that survived the experiment failed to show any gross or histologic evidence of hypoxia. However, animals 62, 58, C-2, and 33 in the control group showed in some degree sufficient pathologic criteria to warrant a diagnosis of periarteritis nodosa with associated acute glomerulonephritis and rheumatic carditis, at least to a mild degree. Valvulitis was not observed in these animals. The remaining animals showed no pathologic change.

TABLE II

(1) Control Group (No Treatment)

Replication	Animal No.	Sensitivity reactions (skin test) Type*	Size Cm X Cm	Precipitin titers at autopsy	Gram weights of each animal throughout the experiment. Loss & gain in body weight—days 1	7	14	21	28	Severity of pathology: Periarteritis nodosa	Rheumatic carditis	Acute glomerulonephritis	Hypoxia
	61	O	O X O	0	1925	2250	2250	2350	2400				
					0	+ 325	+ 325	+ 425	+ 475	0	0	0	0
	37	No – – –	Test	0	2425	2550	2675	2850	2925				
					0	+ 125	+ 250	+ 425	+ 500	0	0	0	0
I	56	O	O X O	1:1,000	1975	2275	2425	2575	2675				
					0	+ 300	+ 450	+ 600	+ 700	0	0	0	0
	62	O	O X O	1:2,000	2575	2650	2775	2850	2875				
					0	+ 75	+ 200	+ 275	+ 300	+	+	0	0
	58	No – – –	Test	0	1825	1975	2175	2550	2625				
					0	+ 150	+ 350	+ 725	+ 800	+	++	+	0
	13	No – – –	Test	0	2475	2525	2800	2750	2775				
					0	+ 50	+ 325	+ 275	+ 300	0	0	0	0
	C-9	O	O X O	1:1,000	2550	2625	2825	2975	3025				
					0	+ 75	+ 275	+ 425	+ 475	0	0	0	0
II	30	No – – –	Test	0	2525	2650	2850	2975	3050				
					0	+ 125	+ 325	+ 450	+ 525	0	0	0	0
	C-5	No – – –	Test	0	2750	2825	3025	3100	3150				
					0	+ 75	+ 275	+ 350	+ 400	0	0	0	0
	C-2	O	O X O	1:2,000	1875	2375	2250	2300	2500				
					0	+ 500	+ 375	+ 425	+ 625	+	+	+	0
	57	No – – –	Test	0	1925	2200	2350	2465	2425				
					0	+ 275	+ 425	+ 540	+ 500	Died on last day of experiment			
	36	O	O X O	0	2475	2850	2925	3000	3150				
					0	+ 375	+ 450	+ 525	+ 675	0	0	0	0
III	53	No – – –	Test	0	2150	2375	2475	2650	....				
					0	+ 225	+ 325	+ 500	...	Died on 26th day of experiment			
	33	O	O X O	1:4,000	2725	2850	2850	3050	3175				
					0	+ 125	+ 125	+ 325	+ 450	++	+	+	0
	55	No	Test	0	2325	2575	2750	2775	2675				
					0	+ 250	+ 425	+ 450	+ 350	0	0	0	0

*Skin sensitivity reactions.
O—No edema. C—Pink center edema.
A—Pink edema. D—Red center edema.
B—Pale edema. E—Necrotic cutaneous edema.

TABLE III

	55	No	Test	0	0	+ 250	+ 425	+ 450	+ 350	0	0	0	0

*Skin sensitivity reactions.
0—No edema. C—Pink center edema.
A—Pink edema. D—Red center edema.
B—Pale edema. E—Purple center edema.

TABLE III

a. Hypersensitivity (Animals Receiving Horse Serum Only)

Replication	Animal No.	Sensitivity reactions (skin test) Type*	Size Cm X Cm	Precipitin titers at autopsy	Gram weights of each animal throughout the experiment Loss & gain in body weight—days 1	7	14	21	28	Periarteritis nodosa	Severity of pathology Rheumatic carditis	Acute glomerulo nephritis	Hypoxia
	41	A	4.5 X 4.0	1:16,000	2700	2825	2900	3075	3012	++++	+++°	++	0
					0	+ 125	+ 200	+ 375	+ 312				
	44	A	4.0 X 5.0	1:8,000	2575	2725	2800	2875	2975	++	+°	+	0
					0	+ 150	+ 225	+ 300	+ 400				
I	C-8	D	5.0 X 5.0	1:8,000	2350	2525	2600	2750	2750	+	+	+	0
					0	+ 175	+ 250	+ 400	+ 400				
	C-7	D	3.8 X 4.2	1:8,000	2850	2900	3075	3250	3400	+	+	+	0
					0	+ 50	+ 225	+ 400	+ 550				
	C-6	A	2.5 X 3.0	1:8,000	2650	2475	2625	2800	3000	++	+++°	+	0
					0	− 175	− 25	+ 150	+ 350				
	63	A	3.0 X 2.5	1:500	2550	2575	2650	2725	2750	+	+	+	0
					0	+ 25	+ 100	+ 175	+ 200				
	64	B	No test	1:500	2575	2650	2750	2950	2950	0	0	0	0
					0	+ 75	+ 175	+ 375	+ 375				
II	48	A	3.3 X 4.0	1:8,000	2700	2800	2925	3000	3050	+++	++°	++	0
					0	+ 100	+ 225	+ 300	+ 350				
	32	C	3.1 X 3.0	1:8,000	2425	1775	1925	2075	1750	+	+	+	0
					0	− 650	− 500	− 350	− 675				
	72	D	4.0 X 5.0	1:8,000	2600	2900	2925	3100	3075	++++	++°	++	0
					0	+ 300	+ 325	+ 500	+ 475				
	28	B	4.0 X 4.2	1:12,000	2375	2350	2625	2725	2950	+	++°	+	0
					0	− 25	+ 250	+ 350	+ 575				
	23	C	2.6 X 4.0	1:14,000	2350	2375	2625	2775	2850	++	++°	++	0
					0	+ 25	+ 275	+ 425	+ 500				
III	C-4	B	3.0 X 3.0	1:10,000	2600	2775	2850	3000	3075	+	+	0	0
					0	+ 175	+ 250	+ 400	+ 475				
	31	B	1.5 X 1.5	1:12,000	2450	2650	2775	2800	2875	+	+	+	0
					0	+ 200	+ 325	+ 350	+ 425				
	25	B	3.2 X 4.1	1:8.000	2550	2750	2850	3000	3100	+	+°	+	0
					0	+ 200	+ 300	+ 450	+ 550				

*Skin sensitivity reactions.
0—No edema. C—Pink center edema.
A—Pink edema. D—Red center edema.
B—Pale edema. E—Purple center edema.

°Mitral valvulitis.
°°Mitral and tricuspid valvulitis.

TABLE IV

b. Animals Receiving Altitude Stress (hypoxia) Only

Replication	Animal No.	Sensitivity reactions (skin test) Type*	Size Cm X Cm	Precipitin titers at autopsy	Gram weights of each animal throughout the experiment. Loss & gain in body weight—days 1	7	14	21	28	Severity of pathology: Periarteritis nodosa	Rheumatic carditis	Acute glomerulo nephritis	Hypoxia
	46	O	O X O	0	2300 0	2450 + 150	2525 + 225	2450 + 150	2600 + 300	0	0	0	+
	39	No - - -	Test	0	2850 0	2925 + 75	3100 + 250	3050 + 200	3225 + 375	++	++°	+	+
I	47	No - - -	Test	0	2650 0	2725 + 75	2750 + 100	2650 0	2825 + 175	0	0	0	+
	76	No - - -	Test	0	2550 0	2725 + 175	2500 − 50	2550 0	2675 + 125	+	++°	+	++
	75	No - - -	Test	0	1975 0	....	....	....	....	..	..	..	..
	26	O	O X O	1:4,000	2350 0	2350 0	2400 + 50	2325 − 25	2475 + 125	0	+	0	++
	10	No - - -	Test	0	2025 0	2325 + 300	2575 + 550	2625 + 600	2825 + 800	0	0	0	+
II	50	O	O X O	0	2075 0	2100 + 25	2250 + 175	2225 + 150	2400 + 325	0	0	0	+
	C-3	No - - -	Test	0	2350 0	2500 + 150	2625 + 275	2625 + 275	2725 + 375	0	0	0	+
	C-1	No - - -	Test	0	2050 0	2250 + 200	2400 + 350	....	....	+	++°	+	+++
	15	No - - -	Test	0	2600 0	2725 + 125	2750 + 150	2800 + 200	3000 + 400	0	0	0	0
	24	O	O X O	1:4,000	2075 0	2125 + 75	2300 + 225	2350 + 275	2475 + 400	+	+°	+	++
III	16	No - - -	Test	0	2175 0	2500 + 325	2650 + 475	2725 + 550	2925 + 750	0	0	0	0
	65	O	O X O	1:2,000	2300 0	2325 + 25	2475 + 175	2400 + 100	2350 + 50	0	+°	0	+
	17	No - - -	Test	0	2000 0	2325 + 325	2500 + 500	2500 + 500	....	+	++°°	+	+++

*Skin sensitivity reactions.
0—No edema. C—Pink center edema.
A—Pink edema. D—Red center edema.
B—Pale edema. E—Purple center edema.

°Mitral valvulitis.
°°Mitral and tricuspid valvulitis.

TABLE V

					0	+ 325	+ 500	+ 500	...				

*Skin sensitivity reactions.
0—No edema. C—Pink center edema.
A—Pink edema. D—Red center edema.
B—Pale edema. E—Purple center edema.

°Mitral valvulitis.
°°Mitral and tricuspid valvulitis.

TABLE V

ab. Hypersensitivity with Altitude Stress (hypoxia)

Replication	Animal No.	Sensitivity reactions (skin test) Type*	Size Cm X Cm	Precipitin titers at autopsy	Gram weights of each animal throughout the experiment. Loss & gain in body weight—days 1	7	14	21	28	Severity of pathology: Periarteritis nodosa	Rheumatic carditis	Acute glomerulo nephritis	Hypoxia
	40	A	4.0 X 5.0	1:10,000	2500	2675	2825	2850	3000	++++	++++°°	++	+++
					0	+ 175	+ 325	+ 350	+ 500				
	42	B	3.5 X 5.0	No test	2775	2875	2750	2650	....	+++	+++°	+++	++++
					0	+ 100	− 25	− 125	...				
I	74	D	3.5 X 4.0	1:8,000	2375	2400	2300	2350	2475	++	++°	++	++
					0	+ 25	− 75	− 25	+ 100				
	11	A	2.5 X 3.5	1:10,000	2525	2325	2550	2575	2850	+	+	++	++
					0	− 200	+ 25	+ 50	+ 325				
	14	B	3.5 X 3.5	No test	2175	2275	2350	....	....	+++++	++++°	+++	++++
					0	+ 100	+ 175	...	...				
	38	B	3.2 X 4.0	1:8,000	2900	2975	3050	3025	3025	++	++°	+	+
					0	+ 75	+ 150	+ 125	+ 125				
	52	A	2.5 X 4.0	1:8,000	2575	2650	2875	2550	3100	+++	++°	++	++
					0	+ 75	+ 300	− 25	+ 525				
II	73	E	2.5 X 2.0	1:6,000	2375	2400	2525	2575	2625	+++++	+++°°	++	+++
					0	+ 25	+ 150	+ 200	+ 250				
	60	C	3.4 X 4.2	1:8,000	2500	2500	2375	2325	2500	+++	++°	+	+
					0	0	− 125	− 175	0				
	59	A	2.5 X 3.5	1:8,000	2425	2475	2525	2550	2650	++	++°	+	++
					0	+ 50	+ 100	+ 125	+ 225				
	34	D	3.0 X 3.0	1:1,000	2300	2425	2375	2375	2550	+	+	0	+
					0	+ 125	+ 75	+ 75	+ 250				
	43	A	3.5 X 2.4	1:8,000	2350	2375	2350	2400	2575	0	0	0	++
					0	+ 25	0	+ 50	+ 225				
III	35	A	2.0 X 3.0	1:10,000	2250	2400	2500	2325	2675	++	+°	++	++
					0	+ 150	+ 250	+ 75	+ 425				
	70	C	3.5 X 4.0	1:8,000	2550	2500	2600	2600	2775	+	+	+	++
					0	− 50	+ 50	+ 50	+ 225				
	51	B	3.5 X 4.0	1:8,000	2425	2525	2600	2600	2800	+	+	+	+
					0	+ 100	+ 175	+ 175	+ 375				

*Skin sensitivity reactions.
0—No edema. C—Pink center edema.
A—Pink edema. D—Red center edema.
B—Pale edema. E—Purple center edema.

°Mitral valvulitis.
°°Mitral and tricuspid valvulitis.

In group *b*, those animals receiving stress only (Table IV), all animals (with the exception of No. 16) presented some gross and histologic evidence of hypoxia. The greatest degree of pathologic change was noted in animals C-1 and 17, both of which died in the altitude chamber on the 20th and 23d day of the experiment, respectively, at an altitude of 26,000 feet. These were autopsied within 1 hour after death. Animal 75 was discarded from the experiment inasmuch as death occurred on the 3d day of the experiment, before stress was received. In 5 animals of this group there was sufficient evidence to warrant the diagnosis of periarteritis nodosa with associated acute glomerulonephritis. Seven cases gave evidence of the existence of rheumatic carditis, and 6 of these exhibited mitral valvulitis either grossly or microscopically. The tricuspid valve was involved in animal 17. Histologically and without culture, these lesions appeared sterile.

TABLE VI

*Quantitative Estimate of Pathologic Injury**
(Test Animals)

	Control (1)	Hypoxia (stress only) (a)	Hypersensitivity (horse serum only) (b)	Hypersensitivity and hypoxia (interaction) (ab)
Periarteritis nodosa	5	6	25	35
Rheumatic carditis	5	11	22	29
Acute glomerulonephritis..	3	5	17	23
Hypoxia	0	19	0	32

*These estimates are derived from the accumulation of relative plus values assigned to the varying degrees of pathologic injury described in Tables 2, 3, 4, and 5.

In the 15 animals receiving horse serum only (Table III), the pathologic changes were more profound. Fourteen showed gross and histologic evidence of periarteritis nodosa with associated acute glomerulonephritis and also rheumatic carditis. Animal 64 was the exception. Eight of the 14 cases showed the presence of mitral valvulitis along with other signs of rheumatic carditis. In animal C-4 no significant kidney lesions were observed, even though this animal exhibited slight periarteritis nodosa and rheumatic carditis.

In animals subjected to both acute altitude stress and horse serum injections (Table V), very significant gross and histologic changes were noted, and on a qualitative basis it was seen that the lesions were very severe.

Hypoxia produced pathologic conditions in all of these cases. Animals 42 and 14 died in the altitude chamber: No. 42 on the 22d day of the experiment at 28,000 feet, and No. 14 on the 20th day at 25,000 feet; periarteritis nodosa, rheumatic carditis, and acute glomerulonephritis were present to a marked degree. Animals 40, 74, 52, 73, and 60 showed the heart and kidney lesions previously described. Animal 43 exhibited no significant gross or histologic evidence of periarteritis nodosa or rheumatic carditis, and the kidney parenchyma failed to show any acute inflammatory process. Ten of the 15 animals exhibited at least mild valvulitis, the lesions, except in 2 cases, being confined to the mitral valves. In 40 and 73, the tricuspid valve was also involved histologically.

In all cases where a pathologic diagnosis of periarteritis nodosa and rheumatic carditis was made, the hearts were grossly enlarged and congested, and showed left ventricular hypertrophy with right ventricular dilatation. The tissue was firm. The kidneys were slightly enlarged, moderately congested, soft, and flabby. No gross infarction was noted.

Discussion

This study was an exploratory one, attempting to point the way to further research on the effects of exposure to one of the stresses of flight (namely, acute altitude stress) on the cardiovascular-renal pathology produced as a result of severe allergic or hypersensitive reactions.

The pathologic manifestations in the experimental animals used in this study were comparable to those described by Rich and Gregory (1943; 1943; 1944) in sensitized animals, and by Highman and Altland (1949) for animals exposed to hypoxia. The pathologic evaluation of the animals employed in these experiments showed that the degree of heart and kidney damage incident to a combination of the protracted hypersensitive state and the additional stress of hypoxia was much

greater than in animals prepared with either the hypersensitive state alone or the hypoxic state alone.

If the fact is accepted that certain aspects of cardiovascular-renal damage, such as periarteritis nodosa with associated acute glomerulonephritis and rheumatic carditis, are manifestations of a severe allergic or hypersensitive state and that certain other aspects of cardiovascular-renal damage are manifestations of hypoxia produced at altitude, it is not difficult to assume that lesions resulting from a combination of these two factors may be of great importance to Air Force personnel. It is well known that such agents as bacteria, viruses, serums, other foreign proteins, and drugs are capable of producing severe allergic or hypersensitive states which can result in local anaphylactic reactions in the tissues of the cardiovascular-renal system.

The temptation to speculate is tremendous, inasmuch as it was shown in this study that one of the stresses associated with flight (namely, exposure to high altitude) increased the lesions produced in hypersensitive or allergic animals. It may be that other environmental stresses peculiar, or pertinent, to Air Force operations will have equal or greater influence on the development of such pathologic changes. The sensitizing capacity of certain propellants, fuels, etc., may be such that personnel not usually affected would be placed in a state of protracted hypersensitivity or severe allergy. An additional stress, such as hypoxia or fatigue, might increase the extent of the damage in these sensitized individuals. It will be interesting to observe whether or not ACTH or cortisone combats the increased pathologic changes resulting from a combination of protracted hypersensitivity and hypoxia, or other stresses. Such a study is in progress and will be reported at a later date.

Summary and Conclusions

The effects of the absence or presence of each of two factors, namely, hypersensitivity to foreign protein and exposure to simulated high altitudes, were observed. Sixty rabbits provided four treatment combinations: 15 were controls; 15 were sensitized to horse serum with no additional treatment; 15 were exposed to simulated high altitude without further treatment;

and 15 were sensitized to horse serum and then exposed to altitude. Qualitative and quantitative estimates of the pathologic lesions were made at the end of 28 days for all four groups of experimental animals.

The pathologic manifestations in animals receiving horse serum alone or those exposed only to altitude were comparable to the manifestations described by other workers. Under the conditions of this study the pathologic evaluation of the animals showed that the degree of heart and kidney damage was much greater with a combination of the protracted hypersensitive state and exposure to simulated high altitude than in the animals prepared with either the hypersensitive state alone or the altitude stress alone.

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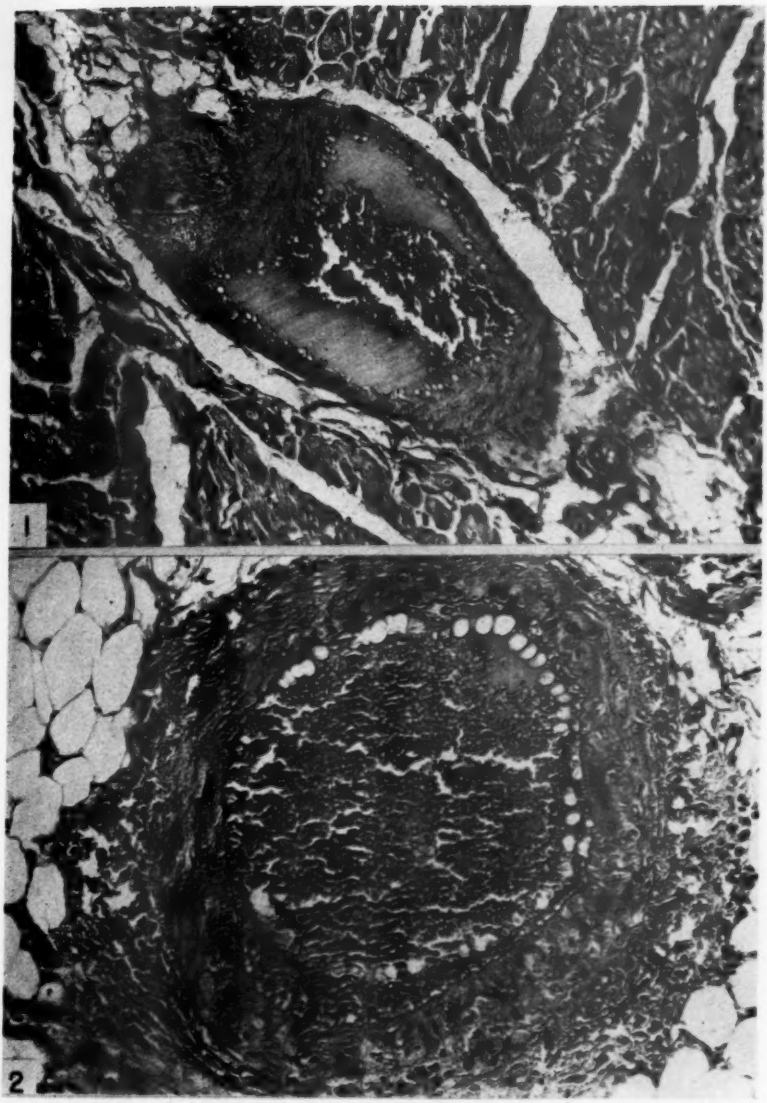

All photomicrographs are taken from sections of rabbit organs of group *ab* (animals sensitized to horse serum and exposed to intermittent hypoxia).

FIG. 1. Heart. Aschoff nodule in wall of coronary artery and showing very early thrombus formation. X 180.

FIG. 2. Heart. Coronary artery showing adventitial and perivascular leukocytic infiltration. Very early intimal sclerosis noted in lower portion of vessel. X 200.

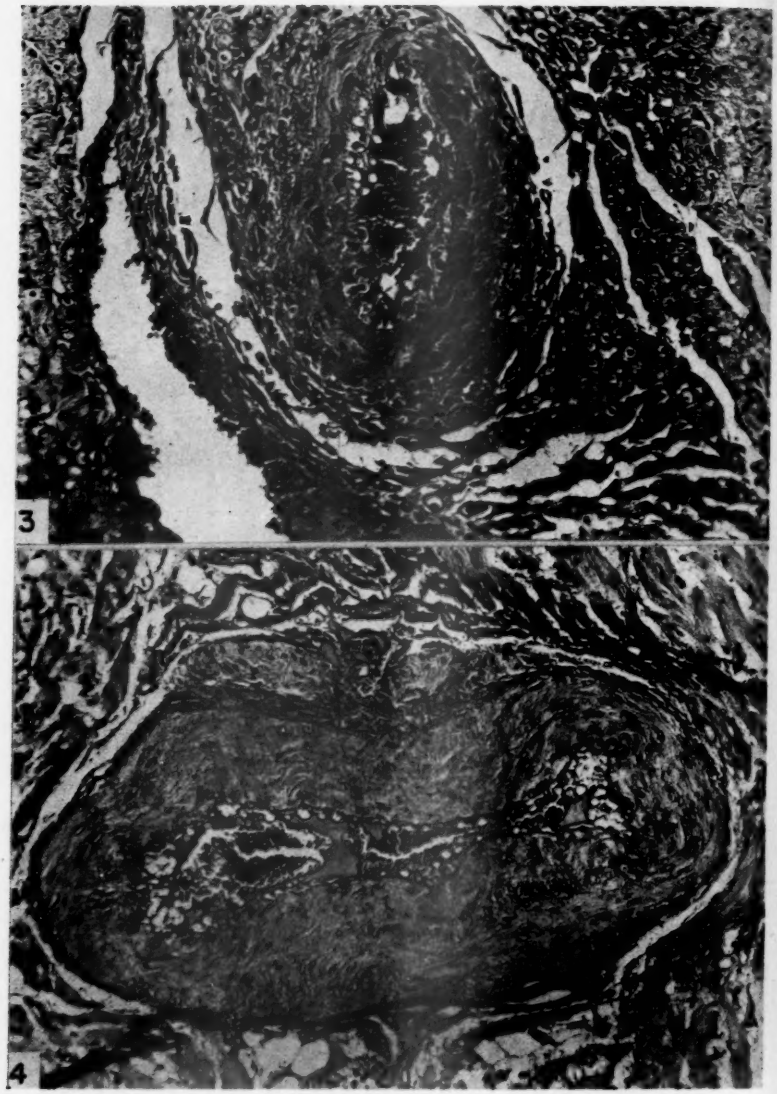

FIG. 3. Heart. Coronary artery showing early intimal and medial thickening with adventitial exudation. X 180.

FIG. 4. Heart. Coronary artery showing intimal thickening, narrowing of the lumen, and nodular formation in the adventitia. X 180.

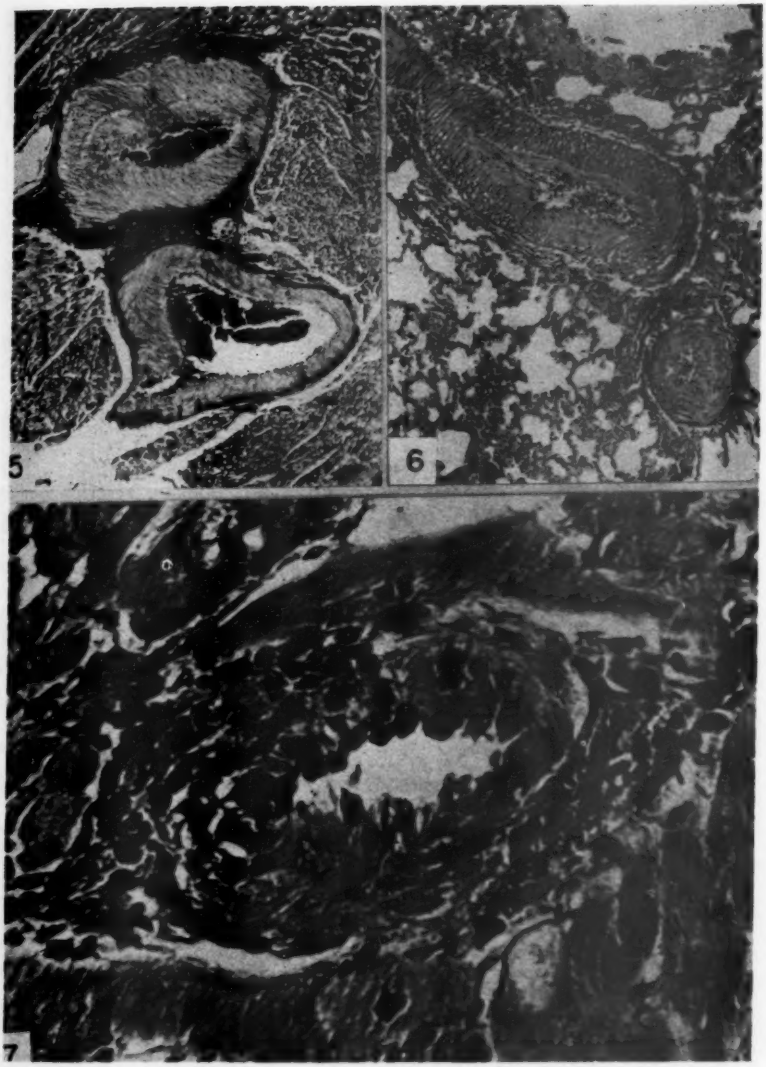

FIG. 5. Heart. Coronary artery showing irregular intimal thickening. X 50.

FIG. 6. Lung. Arteries showing prominent intimal thickening. X 50.

FIG. 7. Heart. Coronary artery showing marked necrosis of all layers. X 625.

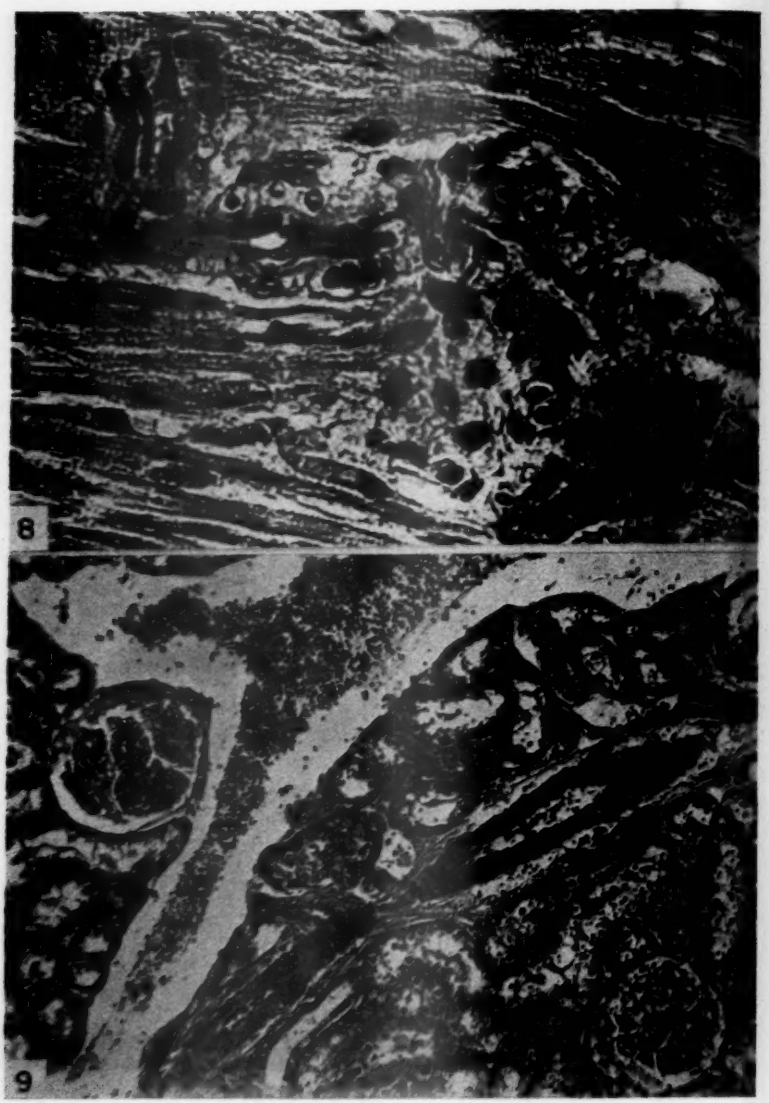

FIG. 8. Heart. Aschoff nodule in myocardium. X 650.

FIG. 9. Kidney. Section showing marked cloudy swelling, endothelial proliferation and leukocytic infiltration in glomerular tufts. Albuminous material in Bowman's capsule and interstitial hemorrhage. X 300.

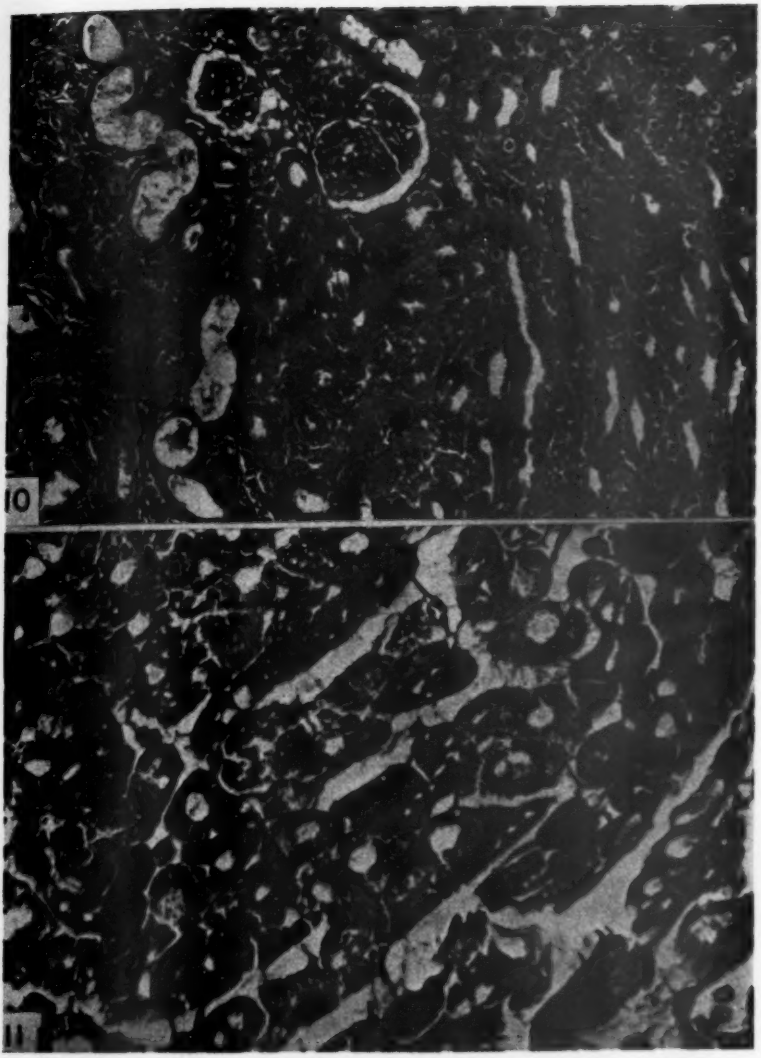

FIG. 10. Kidney. Section showing tubular dilatation, cast formation, tubular necrosis, and interstitial exudation. X 198.

FIG. 11. Heart. Myocardium showing marked vacuolization characteristic of hypoxia. X 850.

CALORIE REQUIREMENTS*

HSIEN WU

Recent advances in our knowledge of vitamins have pushed energy metabolism to the background. At the meeting of the Federation of American Societies of Experimental Biologists, held in Atlantic City last April, 86 papers were read before the American Institute of Nutrition, but none was on energy metabolism. The American Society of Biological Chemists had 374 papers; again, none was on energy metabolism. The American Physiological Society had 469 papers, only three were remotely related to calorie requirements—they were on indirect calorimetry. This lack of interest on energy metabolism is not exceptional. It has been the trend for some time.

However, calorie requirements are still the first consideration in nutrition for most of the people in the world. Freedom from want—one of the four freedoms for which the World War II was fought—means, first of all, freedom from hunger. Freedom from hunger means sufficiency of calories.

Calorie requirements differ from the requirements of other nutrients in an important way. The quantities of carbohydrates, fats, and proteins needed to satisfy the daily calorie requirements are of a *higher order of magnitude* than the quantities of other nutrients required. While the requirements of vitamins and minerals are of the order of milligrams or micrograms, the total requirements in carbohydrates, fats, and proteins are of the order of hundreds of grams.

For this reason, the permissible margin of error, in terms of percentage, in our estimate of calorie requirements is much narrower than that of other nutrient requirements.

For instance, a daily intake of 75 mg. of vitamin C is recommended by the National Research Council. It will make no appreciable difference whether one takes 90 or 60 instead of 75 mg. of vitamin C, that is, 20% above or below the estimate. An excess of 15 mg. of vitamin C is easily disposed of

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by excretion or oxidation, while the effect of a deficiency of 15 mg. is hardly noticeable.

On the other hand, a daily intake of 3,000 calories is, with an ordinary mixed diet, equivalent to about 110 grams of fat and 500 grams of protein and carbohydrate. A 20% excess or deficiency means 600 calories which is equivalent to 67 grams of fat or 150 grams of carbohydrate or protein. An excess of this magnitude cannot be disposed of by excretion, but must be worked off by extra muscular activity or stored in the body as surplus fat. A deficiency of the same magnitude must be made up by drawing upon the tissues of the body with consequent loss of weight, or compensated by a reduction of voluntary activity—which means sluggishness.

It is, therefore, necessary to know calorie requirements more precisely than other nutrient requirements. What do we know about calorie requirements? The general principle governing calorie requirements is well known. Researches on energy metabolism harks back to Lavoisier's experiments at the end of the 18th century. The demonstration of the law of conservation of energy in man goes back to Rubner and Voit, in Germany, and to Atwater in this country toward the end of the 19th century.

The principle is simply this: Intake must be equal to output. But the application of this principle requires knowledge of details which are still lacking.

To be sure, standards of calorie requirements have been proposed in different countries at different times by different authorities. For man doing moderate muscle work the values are, Voit (1880), 3,055 calories; Atwater (1895), 3,500 calories; Lusk (1918), 3,000 calories (net); League of Nations (1935), 3,000 calories; National Research Council (1942–1948), 3,000 calories, to mention only the better known ones. Tables of food composition have been published giving the weight of 100 calorie portions or the caloric value per serving of food. Armed with this knowledge of the standard of calorie requirements and the food table, all that one has to do to maintain caloric balance is make use of them. But actually it is not so simple.

The Food and Agriculture Organization (FAO) of the United Nations, the first aim of which is to assure freedom from hunger

for all the people in the world, convened a meeting of the Committee on Calorie Requirements in September, 1949, in Washington. The task of the Committee was to set up standards of calorie requirements as a basis for the appraisal of food supplies against food requirements and planning food production for the whole world.

The importance of a standard of calorie requirements to the F.A.O. is readily appreciated when it is remembered that the average person consumes 2–3 lbs. of food daily or ⅓ to ½ ton annually. With 2.2 billion mouths to feed in the world, a difference of even only 5% in calorie requirements means many (37–55) million tons of food per year.

As a member of that Committee, I had to review the present status of our knowledge on calorie requirements, and I became aware of the neglect this fundamental aspect of nutrition has fallen into in recent years.

Calorie requirements may be assessed in two ways: Measurement of heat production. The amount of heat produced by the human body is measured directly in a respiration calorimeter or calculated from the amount of carbon dioxide produced and the amount of oxygen consumed.

Measurement of food consumption. When the body weight and composition remain unchanged, the amount of heat produced is equal to the caloric value of the food consumed minus the caloric value of the products excreted.

In the first method, the daily heat production may be broken down into several components for different periods of the day—corresponding to sleeping, walking, working, eating, dressing, etc. The daily heat production is broken down more simply into two components, basal metabolism, the minimum heat production for maintenance of life, and work, additional heat production due to voluntary muscular activity.

Basal metabolism is not as constant as it is supposed to be. It depends intrinsically on body weight and surface and mental state, and extrinsically on diet, and environmental conditions. Reduction of body weight is accompanied by a reduction of basal metabolism. Habitual high protein diet raises basal metabolism, while low protein diet has the opposite effect. Cold climate tends to increase the basal metabolism while

warm climate has the opposite effect. Basal metabolism also increases with nervous tension. Racial difference per se has no appreciable influence on basal metabolism, but the mode of living has a marked influence. Variations of basal metabolism within 10–15% of the standard are usually considered normal. In other words, it cannot be estimated with a greater precision than 15%.

In the first method of assessing calorie requirements, energy expenditure data for different kinds of muscular activity are also needed. Some such data have been obtained—or instance, the energy expenditure per hour of typing, reading, . . . etc. But they are far from being sufficient for the calculation of the calorie requirements of the complex combination of muscular activity involved in a particular occupation. Moreover, the energy expenditure depends on the efficiency with which the work is performed, and efficiency depends in turn on physical build, training, mental state, and environmental conditions.

It is impossible at present to calculate precisely the calorie requirements of an individual from anthropometric measurements and occupation simply on the basis of book knowledge.

In the second method of assessing calorie requirements, knowledge is required of the composition of foods, coefficient of digestibility, and degree of utilization of the major food constituents in each kind of food. Such knowledge is very limited at present.

With regard to food composition, the protein is calculated by multiplying N with the factor 6.25 (corresponding to 16% nitrogen in protein). Substances extracted by petroleum ether are reckoned as fat. Carbohydrate is calculated by difference between the total solid and the sum of protein, fat, and ash. These methods of calculation are only approximate and the difference between the calculated and the actual content for any constituent in a diet may be considerable. For instance, the correct factor for milk protein is 6.38, for refined wheat flour 5.70 instead of 6.25. Not all substances extracted by petroleum ether are fats. "Carbohydrates by difference" may include anything.

As regards coefficient of digestibility and degree of utilization, Atwater took these into account by using the values of 4, 9, and 4 calories for each gram of carbohydrate, fat and protein. These values are lower than the gross caloric values and represent the net physiologically available caloric value of the food constituents. They were determined for the type of mixed diet used in this country in the 90's. If the same factors are applied to other types of diet they may be quite in error because the coefficient of digestibility of a given food constituent in a given food depends not only on the amount of that food but also on the amount of other foods eaten. Moreover, digestibility depends on the method of cooking, mode of living, and the so-called "conditioning factors."

As an example of the magnitude of error involved in using Atwater's average factors for proteins, fats, and carbohydrates, the calculated caloric value of whole wheat is 342 per 100 gram while the actual physiological value as determined by feeding experiment is only 314. It is impossible at present to calculate precisely the heat production of an individual from the food consumption data.

In view of all the uncertainties in estimating the actual heat production and the calorie value of food consumed, it is not surprising that deficiency or excess of the order of 10% or more is a matter of common occurrence.

The Committee on Calorie Requirements of the F.A.O. recommended a method of estimating calorie requirements for groups of peoples—in other words, for the average man and woman, thus avoiding the incalculable individual variations. The method is to take the calorie requirements of a "reference man" or woman as standard and calculate the calorie requirements of any other man or woman by applying correction factors.

The total energy metabolism of a person is known to depend on a number of factors—sex, age, body weight, external temperature, and activity. With the exception of activity, the relation between energy metabolism and each of these factors can be expressed in simple terms.

Sex and weight: $E = 152W^{0.73}$ for man and $E = 123W^{0.73}$ for woman, where E= energy metabolism in calories and W = body weight in kg.

Age: Energy metabolism declines with age. For every 10 years' increase in age, the metabolism decreases by 7.5%.

External temperature: Increase of temperature decreases the energy metabolism. Every 10° C. rise in temperature causes a 5% decrease of energy metabolism.

All these values are based on the best data at present available and are given in round numbers.

"The reference man" is a healthy man, 25 years old, weighing 65 kg., lives in temperate zone at a mean annual external temperature of 10° C., consumes a well-balanced diet and does such muscular work as is involved in an occupation in light industry. Similarly, "the reference woman" is a healthy woman, 25 years old, weighing 55 kg., engaged in general household duties or in light industrial work.

On a "year round" basis, the Committee assigned the values of 3,200 and 2,300 calories for the reference man and woman, respectively. The calorie requirements of any other person whose factors differ from that of the reference man or woman can be calculated by applying the above mentioned correction factors. No correction for activity is applied, on the assumption that in any community those with extremely high or low activity are rare.

The F.A.O. standard is intended for population and not for individuals. But everybody can easily determine his or her own calorie requirements in a simple way. He can do so in terms of *food* instead of calories—without any reference to food composition or theories of nutrition. For the final test of the calorie requirements being satisfied is the constancy of body weight and composition. When this constancy is realized, the calorie requirement is equal to the physiological heat value of the food taken—whatever they may be.

That raises the crucial question: What is the body weight and what is composition (what degree of fatness) that are to be kept constant?

One physiologist has said: "The proper caloric allowance is that which over an extended period will maintain body weight at the level most conducive to well-being." But what is the level most conducive to well-being?

There are three kinds of objectives which may serve as criterion of well-being:

Work efficiency. The object is to perform the maximum amount of a specific kind of work, physical or mental, for a given amount of food intake.

Esthetic appeal. The object is to attain the most beautiful proportion of different parts of the body—taking Apollo and Venus as the standard of perfection.

Health. The object is to attain the maximum health as measured by span of life, fertility rate, morbidity rate, resistance to disease, etc.

The weight or the degree of fatness that is most conducive to the attainment of one objective may not be so for another, and no criterion of well-being has yet been generally agreed upon. Each person has to set his or her own objective and then determine his or her calorie requirements on the basis of that objective.

To do so, one has to weigh the food eaten daily and to check the weight and composition of the body from time to time. Weight is easily taken, but the composition—the degree of fatness—is not so easy to ascertain. For the purpose of research, several methods are available.

The specific gravity of the body may be determined by applying Archimedes' principle. By weighing oneself in air and again in water (for example, in a swimming pool), the specific gravity of the body can be calculated. The greater the amount of fat, the lower is the specific gravity of the body.

X-ray picture of selected parts of the body (*e.g.*, the leg) permits measurement of the thickness of cutaneous and subcutaneous tissue in comparison with those of the bone and muscle.

Water content. The total water content per kg. of body weight decreases with increasing amount of fat.

"Skinfold thickness." The thickness of the double layer of skin together with the subcutaneous fat, may be pinched and measured with a caliper.

These methods, except the last, are beyond the reach of the layman. But he can at least look at himself before a mirror

and that together with weight is sufficient to tell whether his calorie intake is excessive or deficient.

For those who want to contribute toward our knowledge of calorie requirements, there is a simple research which can be done by anybody anywhere with the minimum of cost, labor, or equipment. It is this:

Find in every community those individuals who measure up to the standard of excellence (whatever may be the criterion of well-being).

Record the amount and kind of food eaten daily over a period of months.

Observe his or her mode of living—number of hours spent in each kind of activity or leisure.

From observations such as these, a body of knowledge can be built up which will eventually enable us to set up standards of calorie requirements more precisely than is possible at present.

The science of nutrition is making rapid progress these days. But in the quest for more detail with regard to specific nutrients, let us not lose sight of the fundamental requirement of nutrition—calorie requirements.

SOME SIGNIFICANT ADVANCES IN DIAGNOSTIC BACTERIOLOGY*

E. Staten Wynne

The past few years have witnessed many notable advances in diagnostic bacteriology. It is the purpose of this note to discuss briefly a few of these.

Simplified rapid antibiotic sensitivity tests. Though all, or nearly all, strains of some species of bacteria may be sensitive to a given antibiotic, the spontaneous occurrence and/or development of resistant strains is often encountered. The importance of antibiotic sensitivity tests as a guide to therapy is thus well established.

Older methods of performing sensitivity tests, such as broth dilution or agar diffusion techniques, though accurate, were relatively complex and time-consuming. The work of Bondi *et al.* (1947) and others has led to the development of rapid simple tests using commercially available filter paper discs previously impregnated with a carefully standardized amount of antibiotic.

These tests are performed by streaking clinical specimens rather heavily on the usual isolation plating media and then placing the paper discs containing antibiotic on the inoculated area. If streaking is done carefully, as many as four antibiotics may be tested on one plate. After incubation for 24 hours, the sensitivities of the organism or organisms present are determined from the size of the zones of inhibition surrounding the discs. Sensitivity to antibiotics may thus be determined concurrent with, or even before, identification of etiological agents. If it is necessary to initiate antibiotic therapy before sensitivity tests are completed, the results may be used as an indication to continue therapy or change drugs. Discs

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are now available for a variety of antibiotics including penicillin, streptomycin, dihydrostreptomycin, aureomycin, chloromycetin (chloramphenicol) bactirasin, and terramycin. Discs are also available for several of the sulfa drugs.

Inactivation of antibiotics in clinical specimens used for culture. In cultures taken after institution of antibiotic therapy, especially blood and spinal fluid cultures, the presence of the antibiotic in the material being cultured may prevent growth unless a substance capable of inactivating the antibiotic or neutralizing its effect is incorporated into the medium. Unfortunately our knowledge in this field is rather limited. Sterile preparations of the enzyme penicillinase now available commercially, chemically inactivate penicillin.

Brain heart infusion media are reported capable of nullifying the action of the usual concentrations of streptomycin, presumably due to the presence of lipositol, a component of brain structurally similar to streptomycin (Rhymer *et al.*, 1947). Thus if brain heart infusion media containing penicillinase are routinely employed, penicillin and streptomycin are rendered ineffective. Such media may, of course, be made even more versatile by incorporation of p-aminobenzoic acid to competitively inhibit the action of sulfa drugs which may be present.

Rapid simplified tests for identification of enteric bacteria. A considerable amount of research has been recently directed toward making the identification of enteric pathogens a more rapid procedure, and thus a more valuable diagnostic tool. Knox (1949) developed a screening plate for determining simultaneously the ability of an organism to produce H_2S and to ferment lactose, mannitol, and sucrose. By means of his technique, however, one plate was used for each organism. A method recently devised by Spaur and Wynne (1951) allows the determination of the fermentative abilities of four organisms toward 3 or 4 carbohydrates on a single plate. Strips of filter paper sterilized in concentrated solutions of carbohydrates and dried are placed in the basal agar medium just before solidification and pushed to the bottom of the dish. After solidification, organisms are streaked at right angles to the strips. Production of acid is indicated by a change in color of the phenol red

indicator from red to yellow, and by precipitation of desoxycholic acid, while gas production is determined by fissures produced underneath a sterile cover glass placed over the paper strip containing glucose.

Various rapid tests have been described for biochemical characteristics other than fermentations. Generally, such tests have involved the use of relatively heavy inocula, small amounts of media, and incubation in a water bath. Elek (1948a) reviewed previous attempts to devise a satisfactory rapid urease test, and developed a 3-hour test which seems very promising. Cook (1948) has described a 1½-hour urease test. An indole test giving readings in 2 hours was developed by Arnold and Weaver (1948), while Cook and Knox (1949) described a 1-hour test the following year. A 45-minute test for H_2S production was recently published by Morse and Weaver (1950), who claim that incubation for longer periods makes the test oversensitive.

New differential medium for enteric bacteria. Acid fuchsin methylene blue agar, described by Jeter and Wynne (1949), gives red colonies with lactose fermenters and blue colonies with nonfermenters. Colonies of *Streptococcus faecalis* are not mistaken for nonfermenters and needlessly subcultured, as is often the case with older differential media. The medium is somewhat less toxic than eosin methylene blue agar, particularly for *Proteus* species. In a recent study, Edge and Wynne (1951) have reported acid fuchsin methylene blue agar to be the medium of choice in work involving both isolation and cultivation of *Proteus* strains.

Enrichment media for Salmonella. Though tetrathionate enrichment media have been widely used for *Salmonella*, one difficulty has been the formation of harmful reaction products resulting when iodine is added to convert $Na_2S_2O_3$ to $Na_2S_4O_6$. Pruess (1949a) has recently published a method for preparing pure $K_2S_4O_6$ and a description of its use in tetrathionate broth. A stable agar tetrathionate medium has also been employed by this worker (1949b). A K_3CrO_4 medium containing brilliant green and bile has been reported by Knothe (1949) to give better results than tetrathionate broth in the isolation of *Salmonella schottmuelleri*.

Use of penicillin in isolation of Hemophilus species. In his now classical paper in which penicillin was first described, Fleming (1929) suggested the use of this antibiotic in the isolation of *Hemophilus influenzae.* After the infected material was streaked, 2–6 drops of crude penicillin solution were spread over half of a Petri dish. By means of the growth-suppressing action exerted on accompanying bacteria, isolations of *H. influenzae* from influenza cases were increased from 30% to 90%.

Of considerably more interest at present is a similar use of penicillin in the isolation of *Hemophilus pertussis.* In one of the earliest applications of penicillin, McLean (1937) reported that its use in the cough plate method in a series of 50 specimens increased the number of isolations from 33 to 47. More recently it was found by Bradford *et al.* (1946) that penicillin is of similar value in the nasopharyngeal swab method, which is claimed by these workers to be superior to the cough plate technique. In a series of 95 specimens from 53 patients, use of penicillin increased the number of isolations of *H. pertussis* from 75 to 92.

Cultural and biochemical differentiation of Corynebacterium diphtheriae. By use of a chocolate tellurite agar containing an extract of clotted blood, Welsch *et al.* reported (1946a and 1946b) that it was not only possible to differentiate *C. diphtheriae* strains into gravis, intermedius and mitis types, but that colonies of *C. diphtheriae* could most often be distinguished from those of diphtheroids. The sole exception to this was afforded by mitis type colonies of *C. diphtheriae* and "H" type diphtheroid colonies.

In 1947 Tinsdale noted that colonies of *C. diphtheriae* were surrounded by a clearly defined deep brown halo on a fresh blood tellurite medium containing $Na_2S_2O_3$ and cystine. Halos were either absent or appeared later with colonies of diphtheroids. By addition of $Na_2S_2O_3$ and cystine to the medium of Welsch *et al.*, Thibaut (1948) and Thibaut and Welsch (1948) have been able to secure the Tinsdale phenomenon with *C. diphtheriae* and not with diphtheroids. The differentiation of gravis, intermedius, and mitis types remained, but was somewhat slower. The Tinsdale phenomenon is based on the ability of *C. diphtheriae* to produce H_2S and to acidify a

non-carbohydrate medium, as pointed out by Welsch and Thibaut (1948). While some diphtheroids had the former property, none possessed the latter.

Simple in vitro method of determining toxigenicity of Corynebacterium diphtheriae. An *in vitro* method of determining toxigenicity of *C. diphtheriae* cultures based on the principle of optimal proportions operating in a system employing a double diffusion gradient was first attempted by Ouchterlony (1948) and further developed by Elek (1948b and 1949). Briefly, the method employed by the latter worker involves embedding in a melted serum agar plate a strip of filter paper saturated with a standardized solution of diphtheria antitoxin. After solidification pure cultures to be tested are streaked at right angles to the paper and incubated 24–48 hours.

Since toxin diffuses outward from the line of growth and antitoxin diffuses outward from the paper strip, the points of optimal concentrations for flocculation fall on two continuous lines which are marked by a white precipitate in the agar. If two strains are streaked sufficiently close together, a looping effect occurs. A summation of antigen concentration occurs as the two lines approach each other and a bending of the lines is observed, due to the larger concentration of antigen requiring a correspondingly larger concentration of antibody which will be closer to the filter paper. Fusion will finally occur, since there is only one optimal antibody concentration for a given antigen concentration. Frobisher and co-workers (1951) have published further refinements of Elek's method.

Culture of Brucella. Tryptose has been widely employed in culture media for *Brucella.* However, it was reported by Schuhardt *et al.* (1949) that certain lots of tryptose were toxic to small inocula. Subsequently Rode *et al.* (1950) presented evidence that this toxicity is due to a breakdown product of cystine.

The usual technique of culturing *Brucella* has involved inoculation of broth media, followed by multiple transfers to agar at intervals of 2–4 days for periods up to one month. Furthermore, since repeated cultures have been recommended, the isolation procedure for this group of organisms becomes truly prodigious. In 1947 Castaneda devised a method dispensing

with subcultures. Citrated agar is sterilized in a square bottle and allowed to solidify with the bottle in a horizontal position. Ten ml. of citrated broth medium is then added asceptically. After inoculation with blood, incubation is carried out in the upright position, but the bottles are tilted every other day to allow the blood-broth mixture to flow up over the agar surface. The considerable saving of time effected by the Castaneda technique makes culture for *Brucella* a feasible procedure for the average clinical laboratory.

Recently Pickett and Nelson (1951) reported that washing of citrated blood specimens with distilled water allowed the regular isolation of *Brucella* not only from 50 cases of suspected brucellosis, but also from 100 normal human blood samples. It was felt that the washing procedure removed anti-Brucella bacteriophage and possibly antibodies. Should subsequent work confirm the widespread occurrence of *Brucella* in normal blood, present concepts of brucellosis must be modified.

Blocking antibodies in brucellosis. The fact that occasional patients with proven brucellosis never develop detectable agglutinins, and that agglutinins once formed may disappear rather rapidly in others, has been a puzzling phenomenon. In 1947 Griffitts described the occurrence of agglutinin-blocking antibodies in persons with proven brucellosis and advanced the hypothesis that the blocking action of such antibodies on saline agglutinins may be the cause of failure to detect such agglutinins. When rabbit serum was used instead of saline in performing the test, agglutination regularly occurred. It was reported recently by Cox and Kutner (1950) that a significant number of bovine sera from clinically suspicious herds showing negative or very low agglutinin titers in 0.9% saline exhibited considerable agglutinin-blocking titers.

Rapid mouse test for the laboratory diagnosis of tuberculosis. Injection of guinea pigs with clinical materials containing *Mycobacterium tuberculosis* generally requires a period of several weeks before gross lesions are visible in the spleen, liver, and lymph nodes. A mouse test reported in 1948 by Milzer and Levine yielded grossly visible lesions in 10–15 days. Sputum was mixed with 5% gastric mucin and injected

both subcutaneously and intraperitoneally into special pigmented strains of mice.

Cultivation of Mycobacterium tuberculosis. It was reported by Dubos and Davis in 1946 and confirmed by other workers that media containing Tween 80 may not give visible growth from minute inocula. (See Gernez-Rieux *et al.*, 1948a for review.) It has been suggested by Gernez-Rieux *et al.* (1948b) that the toxic action of such media may be due to an inhibitory factor liberated by the developing *M. tuberculosis* cells before their number becomes sufficiently large for ready detection. It was shown by the same workers (1948a and 1948c) that this toxicity may be neutralized by the addition of cholesterol or potato extract. The same year Málkova and Málek (1948) achieved similar results with added 1% soluble starch.

Dubos and Middlebrook (1947) have described an agar medium containing oleic acid and albumin. Virulent strains of *Mycobacterium tuberculosis* are distinguished from avirulent strains and other bacteria by their characteristic cord-like structure under the low power lens (Middlebrook *et al.*, 1947). By the addition of 100 units of penicillin per ml., Kirby and Dubos (1947) were able to inhibit contaminants without affecting the growth of the tubercle organism. Smith *et al.* (1949) have reported that this medium was slightly more sensitive than a standard egg-potato medium in the detection of *Mycobacterium tuberculosis* from clinical materials. Recently Byham (1950) found Middlebrook agar (*cf.* Middlebrook, 1947) to be roughly twice as effective as Dorsett's egg and Petroff's egg media in isolations from sputums and gastric washings. The medium was particularly valuable in clinically questionable cases.

An interesting development in the cultivation of *Mycobacterium tuberculosis* was reported very recently by Tarshis and Frisch (1951). It was found that ordinary blood agar base medium with 25% added human blood and 1% glycerine was at least equal to, and in many cases better than, three standardized media (Petragnani's, Lowenstein's, and Corper-Cohn). Citrated, oxalated, and defibrinated blood were equally satisfactory. Discarded blood from a blood bank was entirely suitable, even when 56 days old.

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CALLING ATTENTION TO:

Items of Possible Interest to Our Friends

September, 1951

Preclinical: BR Boone & Co edit conference on electrokymography (Publ Health Service Publ 59, Washington, 51, 215pp, 75c). C Carruthers & V Suntzeff show common biochemical properties of tumors from different tissues (*Science* 114/103/July 27/51). VH Cicardo & Co find pituitary inhibition by ultrasonic irradiation (*Nature* 168/169/July 28/51); *Rev Soc Argentina Biol* 27/43/50/51). JW Cornforth & Co find antituberculous effect of surface active polyoxyehtylene ethers (*Nature* 168/150/July 28/51). JT Edsall edits *Enzymes & Enzyme Systems* (Harvard Univ Press Cambridge, Mass, 51, 146pp, $2.75). N Emmelin shows histamine destroyed in kidney with some appearing in urine (*Acta Physiol Scand* 22/379/51). H Haimovici & Co find phenoxy-isopropyl benzyl-chlorethylamine useful in hypertension & vascular disease (*Proc Soc Exp Biol Med* 77/477/51). JB Gatenby & TAA Moussa describe senility clinkers in human sympathetic neurones (*J Physiol* 114/252/51). R Gaunt & JH Birnie discuss *Hormones & Body Water* (CC Thomas, Springfield, Ill, 51, 57pp, $2). RD Keynes supports sodium hypothesis of nerve action potential from membrane permeability changes (*J Physiol* 114/119/51). F Koller & Co isolate another clotting factor (*Acta Haematol* 6/1/51). AI Lansing reviews physiological aspects of ageing (*Physiol Rev* 31/274/51). CE Lumsden well describes neurite outgrowth in tissue culture (*Anat Rec* 110/145/51). CN Mangieri & Co find synthetic anticoagulant "treburon", a polygalacturonic acid glycoside, acts like heparin (*J Pharmacol* 102/156/51). KO Moller offers *Rauschgifte & Genussmittel* (B Schwabe, Basle, 51, 432pp, SwFr 19.5). H Nakamura describes pleolysis of red cells (*Yokohama Med Bull* 1/65/51). M Reif & R Wiesman propose chlorphenyl oxycumarin as effective rodenticide (*Acta Trop* 8/97/51). SRM Reynolds analyses determinants of uterine growth & activity (*Physiol Rev* 31/244/51). O Secker shows muscle relaxation under ether due to blocking motor end plate (*Acta Pharmacol Toxicol* 7/119/51). K Sommermyer reviews target theory of radiation biology (*Naturwiss* 18/289/51). A Taylor & N Carmichael find folic acid analogs inhibit tumor growth (*Cancer Res* 11/519/51). H Werner, A Freud, RR Sears & LK Frank contribute to Clark Univ Symposium on genetic psychology (*Am J Orthopsych* 21/472–522/51). VP Whittaker discusses cholinesterases (*Physiol Rev* 31/312/51).

Clinical: WB Bean & Co show tryptophane aids in pellagrous glossitis (*J Lab Clin Med* 38/167/51). In finding increased arterial supply to skin cancer, HR Bierman & Co note intrarterial injections of nitrogen mustard helpful (*J Nat Cancer Inst* 11/877, 891/51). WL Bostick & L Hanna revive virus etiology of Hodgkins disease (*Cancer Res* 11/505/51). JH Burn ascribes antidiuretic action of nicotine from smoking to release of vasopressin from post-pituitary, which also constricts coronaries (*Brit Med J* 2/202/July 28/51). JV Dacie discusses auto-antibodies in hemolytic disease (*Rev Hematol* 6/267/51). JB Firstbrook recommends control of obesity

to prevent atherosclerosis (*Brit Med J* 2/133/July 21/51). BS Hetzel & DC Hine suggest helpful effect of salicylates in arthritis due to activation of pituitary adrenal cortisone production (*Lancet* 2/94/July 21/51). V Lunn shows electroshock followed by rise in skin temperature due to capillary dilation (*Acta Psych Neurol Scand* 26/7/51). F Magrassi & Co suggest viral etiology for leukemias (*Acta Haematol* 6/38/51). FH Mayfield well discusses *Causalgia* (CC Thomas, Sprinfield, Ill, 51, 67pp, $2). RR Overman reviews Na, K & Cl alterations in disease (*Physiol Rev* 31/285/51). LM Randall & TW McElin discuss *Amenorrhea* (CC Thomas, Springfield, Ill, 51, 74pp, $2). L Rangell describes role of emotions in cardiovascular disorders (*Ann West Med Surg* 5/610/51). GM Roth analyses *Tobacco & the Cardiovascular System* (CC Thomas, Springfield, Ill, 51, 66pp, $2). A Simon surveys psychological problems of ageing (*Calif Med* 75/73/51). N Taylor & Co confirm (*Am J Med Sci* 222/138/51) MM Stanley & EB Astwood's report (*Endocrin* 41/66/47) on antithyroid action of methyl mercaptoimidazol (Methimazole). JHW van Ophuijsen (1882–1950) & Co sympose on endocrines & histamine in psychiatric disorder (*J Clin Exp Psychopath* 12/1/51). P Zimmering & Co discuss heroin addiction in NY adolescents (*J Nerv Ment Dis* 114/19/51).

General: JL Baughman reflects on climate, cattle & cross-breeding (*Texas J Sci* 3/253/51). E Bergler discusses *Money & Emotional Conflicts* (Doubleday, NY, 51, 269pp, $3.5). AW Downie philosophizes on Jenner's cowpos inoculation (*Brit Med J* 2/251/Aug 4/51). JH Hildebrand discusses chemistry and education (*Chem Eng News* 29/3279/Aug 13/51). WR Thrower notes medical contributions of Capt James Cook (1728–1779) (*Lancet* 2/215/Aug 4/51).

October, 1951

Of Broad Interest: L Belloni incites wonder at drei hodened B Colleoni (1400–1475) (*Centaurus* 1/43/50). JD Bernal brings TH Huxley up to date in *The Physical Basis of Life* (Routledge, London, 51, 80pp, 6s). EF Caldin questions implications & presupposition in relation of science to philosophy (*Brit J Phil Sci* 1/196/51). Sir Henry Dale & Co sympose well on measurement in medicine (*Brit Med Bull* 7/261–327/51). JC Eccles discusses hypotheses relating to brain-mind problem (*Nature* 168/53/July 14/51). M Fishbein suggests ways of meeting problem of medical indexing (*PG Med* 10/165/51). MF Farnham treats of *The Adolescent* (Harper, NY, 51, 243pp, $3). AJ Liebling comments on varied press accounts of WR Hearst (1862–1951) (*New Yorker*, Sept 8/51/60). CW Mills looks at *White Collar: The American Middle Class* (Oxford Univ Press, NY, 51, 378pp, $5). N Mumey describes the peyote ceremony among Amerinds (*Bull Med Lib Asso* 39/182/51). *The Education of Man* by H. Pestalozzi (1746–1827) finally appears (Philosoph Libr, NY, 51, 93pp, $2.75). Sir John Parkinson discourses on patient-physician relation (*Ann Int Med* 35/307/51). E Schrodinger reflects on *Science & Humanism: Physics in Our Time* (Cambridge Univ Press, 51, 141pp, 7s6d). HE Sigerist receives greetings on his 60th (*Gesnerus* 8/1–194/51). So does B Zondek (*Acta Endocrinol* 7/3–387/51).

Of Clinical Concern: GS Barrett says alumina gels increase stool phosphate excretion thus reducing tendency to urinary calculi (*J Urol* 66/315/51). GE Burch & CT Ray review mechanism of congestive heart failure (*Am Heart J* 41/918/51). E Denhoff & RH Holden discuss pediatric aspects of cerebral palsy (*J Pedia* 39/363/51). EM Glaser & RVH Jones find shivering initiated by cooled blood returning from legs (*J Physiol* 114/277/51). W Hadorn & G Riva analyse clinical significance of disturbances of potassium metabolism (*Schw Med Wchnschr* 81/761/Aug 11/51). BA Houssay gives Ciba Foundation Lecture on sex hormones in diabetes (*Brit Med J* 2/505/Sept 1/51). K Iversen & E Meulengracht treat polycythemia with oral naphthyl dichlorethylamine (*Brit Med J* 2/510/Sept 1/51). JA Keen proposed standardized method of estimating cranial capacity (*S Afr J Clin Sci* 2/170/51). H Koenigstein claims high K & low Ca in cerebrospinal fluid cause pruritus (*J Invest Dermatol* 17/99/51). W Mayer-Gross describes psychoses from mescaline & lysergic diethylamide (*Brit Med J* 2/317/Aug 11/51). EL McCawley, GA Weston & NA David screen antihistamines for use in auricular fibrillation & select diphenhydramine as better than quinidine (*J Pharmacol* 102/250/51). O Moller describes dermatographic lymphadenitis (*Acta Path Microb Scand* 28/352/51). J Reid & Co suggest easy chelate ring formation as factor in salicylate action & find Na resorcylate effective in rheumatic fever (*Brit Med J* 2/321/Aug 11/51). WA Reilly surveys the overweight child (*GP* 4/41/51). W Sargant well describes mechanism of "conversion" (*Brit Med J* 2/311/Aug 11/51). WW Spink defines chronic brucellosis (*Ann Int Med* 35/358/51). S Taylor & FS Stewart note method of estimating iodine distribution in thyroid (*Lancet* 2/232/Aug 11/51). J Zahorsky's report on herpangina, fever, sore throat & tonsillar ulcers in children in summer (*South Med J* 13/871/1920) is found to be caused by Coxsackie viruses (RH Parrott & Co., *New Eng J Med* 275/245/Aug 23/51). G Zetler finds histamine hastens blood coagulation & antihistamines delay it (*Arch exp Path Pharmakol* 213/18/51).

Of Preclinical Interest: A Albert offers *Selective Toxicity* with reference to chemotherapy (Methuen, London, 51, 210pp, 7s6d). I Costero notes peculiar reaction of microglia in rheumatic brains, suggested by TG Perrin to be called "nodule of Costero" (*Gac Med Mexico* 81/49/51). H Croxatto & Co note formation of anti-diuretic agent from pepsitensin (*Acta Physiol Latino-Amer* 1/178/51). A Divry doubts anoxemic theory of renal hypertension & suggests stimulation of presso-sensitive mechanism released at 60mm Hg pressure at entrance to organ (*Arch Internat Physiol* 59/211/51). JA Herschfus & Co survey drugs for relief of bronchial asthmas & find both anticholinergic & antihistaminic action needed for success (*Internat Arch All App Immun* 2/97/51). D Kodlin suggests application of analysis of covarience in pharmacological problems (*Arch Internat Pharmaco* 87/207/51). E Pollard & Co note inactivation of pepsin & trypsin by electron bombardment (*Arch Biochem Biophys* 33/9/51). H Teir & Co find growth promoting factor in skin effective on IP injection (*Ann Clin Gyn Fenn* 40/51, 61/51). EM Widdowson & CM Spray analyse fetal chemistry (*Arch Dis Child* 26/205/51).

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